RESEARCH PAPER

Is sub-chronic exercise in Combination with medicinal nanoparticles a protective strategy against Doxorubicin-induced Hepatic oxidative stress and apoptosis in aging model rats?

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Abstract

Objective(s): Oxidative stress and apoptosis are the major side effects of doxorubicin (DOX) and the advantages accruing from exercise and some medicinal herbs in mitigation of these toxic side effects is well documented. But so far, the effects of exercise in combination with medicinal nanoparticles on oxidative stress and apoptosis signaling simultaneously, in liver tissue are unknown. Hence, we investigated whether Treadmill Running in combination with Nanocurcumin protects the liver tissue against these toxic side effects (oxidative stress and apoptosis) simultaneously of DOX treatment in aging rats induced by D-galactose.

Materials and Methods: Fifty-six Wistar male rats received a daily injection of D-galactose (100 mg/kg/day, i.p.) then randomly assigned to 7 sub-groups. The training protocol included treadmill running progressively between 25 to 54 min/day and 15 to 20m/min, 5 days/week for six weeks. DOX (1 mgmL−1kg−1day−1) was administrated intraperitoneally for 15 days and Nanocurcumin was administrated orally for 2 weeks (100 mg/kg/day).

Results: Nanocurcumin Consumption led to insignificant increase in SOD, MDA and insignificant decrease in AIF levels. Treadmill running led to insignificant increase in SOD and insignificant decrease in AIF and MDA levels. The combination of Treadmill running and Nanocurcumin led to significant decrease in MDA and insignificant increase in SOD and insignificant decrease in AIF levels.

Conclusion: In conclusion, Treadmill running exercise and Nanocurcumin partly mitigates the toxic side effects of DOX treatment. But this amount of treatment does not play a required role against DOX-induced hepatic damage.

Keywords: *Animal models, Apoptosis, Nanoparticles, Oxidative stress, Physical Activity*

How to cite this article

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INTRODUCTION

Doxorubicin (DOX) is a highly effective anti-neoplastic agent, which used in the treatment of several human neoplasms; however, its use in clinical chemotherapy is limited by a dose-dependent toxicity in non-target tissues, including the liver [1, 2]. The mechanisms responsible for DOX-induced toxicity are not clearly known. Several explanations have been proposed to account for DOX toxicity such as free radical formation that leading to lipid peroxidation, calcium overloading, mitochondrial dysfunction and eventually, programmed cellular death, apoptosis [3, 4].

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Among the strategies proposed as effective in counteracting the hepatotoxicity associated with DOX treatment, physical exercise has been recommended as a non-pharmacological tool against liver injury [4, 5]. Previous work suggested the advantage of acute, sub-chronic and chronic exercise models on triggering a preconditioning-like effect on DOX-treated rats [5,6]. In fact, it is well described that endurance exercise training improves hepatic tolerance to deleterious stimuli that cause intracellular oxidative stress and apoptosis [4].

The role of reactive oxygen species (ROS) in DOX-induced toxicity is also supported by the findings that several antioxidants protect organs

Table 1. Subject's weight status during study protocol

Groups	Parameter Body weight at the start of study (g)	Body weight at DOX injection (g)	Body weight at sacrifice (g)	Liver weight (g)	Liver weight/Body weight
S+D	265.7±43.6	326.1±47.6	280.4±47.7*	11.5±3.1	0.0416±0.01
S+P	262.3±43.9	340.4±53.5	381.9±64.1	14.2±3	0.0364±0.00
S+NaCu+D	257.1±52.8	323.4±66.5	260.9±29.4*	10.7±1.93	0.0408±0.00
S+NaCu+P	258.8±48.8	345.1±72.2	378.3±74.3	13.8±3.3	0.0849±0.14
T+D	267.2±27.6	333.7±22.9	265.3±33.6*	11.1±2.37	0.0411±0.00
T+P	272.8±27.7	339.4±40	369.5±32.7	12±2.4	0.0319±0.00
T +NaCu +D	274.7±26	348±21.5	294.8±26*	12.6±2.33	0.0419±0.00

Values are means± S.E.M., P<0.05 compared with the placebo groups. Abbreviations: S+D (sedentary + DOX), S+P (sedentary + placebo), S+NaCu+D (sedentary + Nanocurcumin + DOX), S+NaCu+P (sedentary + Nanocurcumin + placebo), T+D (Training + DOX), T+P (Training + placebo), T +NaCu +D (Training + Nanocurcumin + DOX)

Table 2. Effects of DOX treatment, treadmill running and Nanocurcumin Consumption on MDA, SOD and AIF levels in the various groups

Groups	Markers MDA (μM)	SOD (U/ml)	AIF (ng/ml)
S+D	9.53±2.5	50.3±8.1	68.4±8.2
S+P	7.93±2.13	58.4±13.1	74.2±9.7
S+NaCu+D	10.81±1.43	53.5±5.5	68.5±5.9
S+NaCu+P	7.73±1.93	41±5.5	78.8±18
T+D	7.43±2.61	51.8±5.3	77±10.7
T+P	5.28±1.87	51.7±9.8	82.8±11.3
T +NaCu +D	8.63±2.60*	44.4±8.1	75.8±4.4

Values are means± S.E.M., P<0.05 compared with the S+D group. Abbreviations: Malondialdehyde (MDA), Superoxide dismutase (SOD), Apoptosis Inducing Factor (AIF). S+D (sedentary + DOX), S+P (sedentary + placebo), S+NaCu+D (sedentary + Nanocurcumin + DOX), S+NaCu+P (sedentary + Nanocurcumin + placebo), T+D (Training + DOX), T+P (Training + placebo), T +NaCu +D (Training + Nanocurcumin + DOX)

against the toxicity of DOX. Therefore, the protective potential of several antioxidant compounds have been investigated in DOX-induced organ toxicity [2, 3, 7]. Some plant products have also been reported to cause augmentation of cell antioxidants. A more recent discovery is Curcumin {1, 7-bis (4-hydroxy-3 methoxyphenyl) - 1, 6- heptadiene-3, 5-Dione} (diferuloyl methane), the principle coloring agent present in the rhizomes of Curcuma longa (zingiberaceae) [8]. Previous work has shown that, Curcumin controls inflammation, cell growth and apoptosis, being thus useful to prevent and treat some diseases thanks to its anti-oxidant and anti-inflammatory activities and excellent safety profile [9]. It has been reported that, it has multiple therapeutic activities that block the hepatic and renal toxicities induced by DOX and it also possibly acts as a free radical scavenger [10,11].

However, from many pharmacokinetic studies, it can be deduced that oral bioavailability of Curcumin is rather poor, which would certainly put some boundaries in the employment of this chemical. This may be due to low absorption, rapid metabolism and elimination, and limited systemic bioavailability [12]. Consequently, for any developments of Curcumin in future, analogues of Curcumin which have better bioavailability or substituted formulations are crucial. To prevail over these weak points, different ways were tested such as piperine, liposomal Curcumin, and nanoparticle Curcumin use [12, 13]. In pharmaceutical products, the most effective process for stabilizing and improving bioavailability Curcumin is nanoparticles. Recent studies suggested that Curcumin nanoparticles enhance the solubility of Curcumin by improving bioavailability more than 5 times from simple Curcumin powder [14, 15].

It was also reported that, chronic D-galactose (D-gal) exposure could induce memory loss, neurodegeneration, oxidative damage and impair neurogenesis in the dentate gyrus, a process similar to the natural aging in mouse. Rodent chronically injected with D-gal has been used as an animal aging model for anti-aging research and health food testing. Moreover, it is well known that, Cancer is a disease of aging as older adults are much more likely to develop cancer compared with their younger counterparts [16]. Clinical studies have also shown that, with the aging increasing, the anti-oxidative defense systems include enzymes such as superoxide dismutase (SOD), decline during the aging process. Oxidative stress plays an important role during the pathogenesis of aging and age-associated diseases such as cancers [17]. In contrast,

recent studies focused only on the young subjects in their works [1,2,3,4,5]. Based on the reasons above, in this study, aging model rats were established by injecting the male Wistar rats intraperitoneally with D-gal once a day. Also, since aging is associated with age-related muscle weakness [18], and maximal aerobic capacity in regularly exercising decreases with aging [19], and since patients that receive DOX treatment often experience severe fatigue and their ability to engage in high intensity and high volume training is reduced; therefore we prescribed relatively low-dose endurance exercise for subjects.

To the best of our knowledge, concomitant effects of exercise training and medicinal nanoparticles, particularly Nanocurcumin on DOX-induced toxicity in the liver are still unknown. Furthermore, so far, no effective and clinically applicable treatment is yet discovered to prevent DOX-induced hepatic damage in aging. Hence, in the present study we asked for exploring the role of Lipid peroxidation (MDA), antioxidants enzymes such as super oxide dismutase (SOD) and Apoptosis Inducing Factor (AIF), as well as the apoptotic marker in DOX-induced hepatotoxicity in aging induced by D-gal. In addition, we sought to evaluate the possible protective effects of regular Treadmill Running in combination with Nanocurcumin supplementation against DOX-induced oxidative stress and apoptosis signaling, simultaneously in aging model rat liver.

MATERIAL AND METHOD

Experimental design and laboratory environment

This experimental study was done in Faculty of Physical Education and Sport Sciences, Department of Sport Physiology, University of Mazandaran, Babolsar, Iran. This study was approved by department of physiology, university of Mazandaran were performed according to guiding procedures in the care and use of animals, prepared by the Council of the American Physiological Society. The experiments were carried out with fifty-six Wistar male rats, (10-weeks-old, initially weighing 266±36.63 g), which were obtained from the laboratory of animal bearing and multiplying at the Pasture institute of Iran. Rats were housed in standard cages of polycarbonate (20 × 15 × 15 cm), made at the Pasture institute of Iran, in a large air conditioned room with a controlled temperature of 22 ± 2°C, light dark cycles of 12: 12 h and humidity of 50 ± 5%. They were given standard rat chow (Pars Institute for animals and poultry) and water ad libitum. The rats were observed daily and body weights were recorded weekly.

MATERIALS

D-gal was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Doxorubicin hydrochloride was purchased from Pfizer Co., Australia. Nanocurcumin were obtained from Exir Nano Sina Co., Iran. Enzyme-Linked Immunosorbent Assay (ELISA) kits; Malondialdehyde (MDA), Colorimetric, ZellBio GmbH, Ulm, Germany Sensitivity: 0.1 µM. Super Oxide Dismutase activity, Colorimetric, ZellBio GmbH, Ulm, Germany, Sensitivity: 1 U/ml and Rat Apoptosis inducing factor, ELISA, ZellBio GmbH, Ulm, Germany, Intraassay CV% : 3.3, Sensitivity: 1 ng/ml.

Familiarization and Subjects classification

Animals were habituated to new condition for two week. Then, they were adapted to the treadmill by running for 5 days. The familiarization protocol was designed as once a day for 5-10 min/session at a speed of 5-8 m/min at a slope of 0 degree. At the rear of the lines, an electric grid provided a stimulus for running. An electric stimulus (30 V and 0.5 A) was manually turned on for less than 2 s when the animals stayed on the electric grid for longer than 10 s. Rats quickly learned to stay on the belt and avoid shock. Following this familiarization period, all subjects were randomly assigned into seven groups of eight rats each (see Fig 1). The groups were defined as follows:

Group 1; sedentary + DOX (S+D, n=8): The rats were exposed to D-gal melted in normal saline

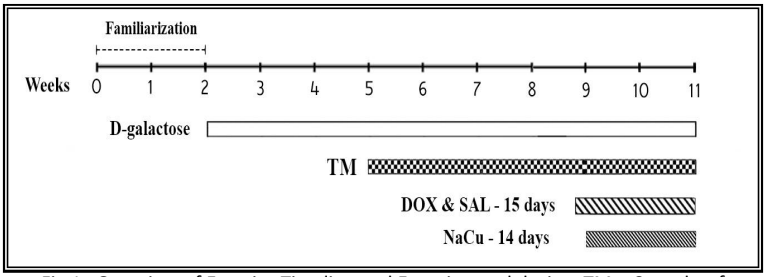

Fig 1. Overview of Exercise Timeline and Experimental design. TM = 6 weeks of treadmill exercise; SAL = saline injection; DOX= doxorubicin injection; NaCu = Nanocurcumin supplementation

(0.9% saline and distilled water) at a concentration of 100 mg/kg intraperitoneally, daily for 9 weeks, in order to induce aging [20]. Also, they were received either daily 1 mg/kg intraperitoneal DOX injections administered over the course of 15 consecutive days [21]. In the present study, we used a cumulative dose of DOX for induce of hepato-toxicity, because to our knowledge, majority of studies have focused on acute toxicities following a bolus DOX dose, whereas we concentrated on early chronic toxicity in aged rats. It is reported that both the cumulative dose and low or high age at the time of treatment are the major risk factors for increased DOX-induced hepato-toxicity [3, 5].

Group 2; sedentary + placebo (S+P, n=8): The rats in this group similarly received D-gal and they were received daily 1 mg/kg intraperitoneal injections of 0.9% saline over the course of 15 consecutive days.

Group 3; sedentary + Nanocurcumin + DOX (S+NaCu+D): The rats in this group received D-gal and DOX similar to that in group 1, as well as In the last 14 days of study protocol, Animals assigned to this group received either daily 100 mg Curcumin/kg in nanoparticle-encapsulated form by oral gavage.

Group 4; sedentary + Nanocurcumin + placebo (S+NaCu+P): The rats in this group received D-gal and Nanocurcumin similar to that in group 3, and in addition, received 0.9% saline similar to that in group 2.

Group 5; Training + DOX (T+D): The rats in this group received D-gal and DOX similar to that in group 1; in addition they performed progressive running exercise on treadmill with zero slopes between 25-54 min.session^{-1} and 15 to 20 m.min^{-1}, 5 days per week for 6 weeks; The running speed and duration of exercise were progressively increased during a graded treadmill exercise protocol. We replicated the aforesaid exercise training protocol that was previously reported by Zolfagharzadeh and Roshan (2013).

Group 6; Training + placebo (T+P): The rats in this group received D-gal and performed Treadmill Running protocol similar to that in group 5. Also, they were received 0.9% saline similar to that in group 2.

Group 7; Training + Nanocurcumin + DOX (T +NaCu +D): The rats in this group received D-gal, DOX, Nanocurcumin similar to that in group 3 and they performed Treadmill Running protocol similar to that in group 5.

Liver tissue collection and preparation

Rats in all groups were anesthetized with ketamine and xylazine following 48h after interventions and 12 h fasting. The abdominal cavity was opened to expose the liver tissue. Then liver tissue were rapidly excised, rinsed, carefully dried, weighed and it was placed into Petri dishes containing cold isolation medium (0.1 mol/L K_2HPO_4, 0.15 mol/L NaCl, pH 7.4) to remove the remaining blood and were frozen immediately in liquid nitrogen and stored at -80ºC, for subsequent analysis of MDA, SOD and AIF. Then, 0.1 g Liver tissue was squashed in liquid nitrogen, homogenized in 1 ml phosphate-buffered saline (PBS) including; 8g NaCl, 0.201g KCl, 1.419g Na_2HPO_4, 0.244g KH_2PO_4 and 800ml distilled water, pH 7.2 and the filtrate was collected. These samples were first centrifuged by a refrigerated centrifuge at 5,000 rpm at 2-8ºC for 15 minutes, immediately after collection and then stored at -80 C, before assay biochemical estimations. Apoptosis-inducing factor (AIF) was measured by ELISA method following the manufacturer's instructions, as described by Bajt [22]. Oxidative stress parameters such as; superoxide dismutase (SOD) was determined at

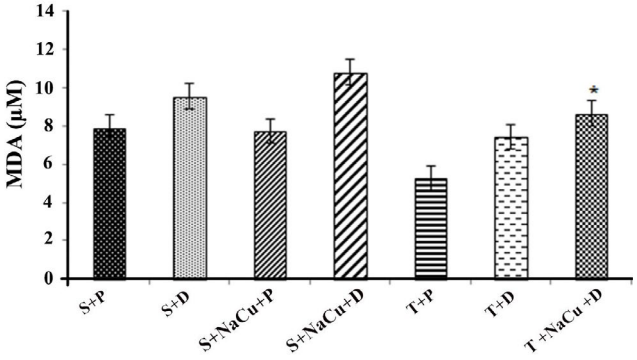

Fig 2. Malondialdehyde (MDA) level after six weeks of Treadmill Running, DOX treatment and Nanocurcumin supplementation. Abbreviations; S+D (sedentary + DOX), S+P (sedentary + placebo), S+NaCu+D (sedentary + Nanocurcumin + DOX), S+NaCu+P (sedentary + Nanocurcumin + placebo), T+D (Training + DOX), T+P (Training + placebo), T +NaCu +D (Training + Nanocurcumin + DOX).
* Significantly different to the S + D group ($P < 0.05$)

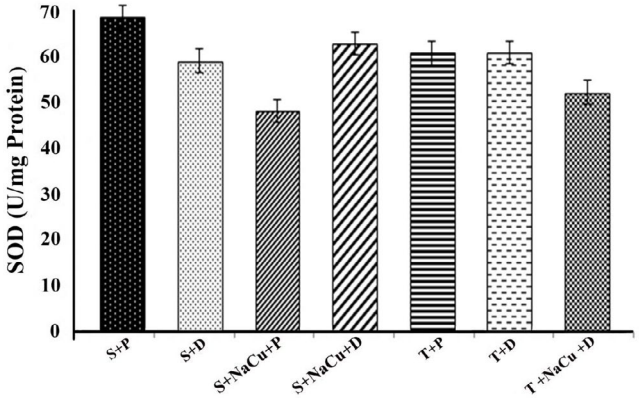

Fig 3. Superoxide dismutase (SOD) level after six weeks of Treadmill Running, DOX treatment and Nanocurcumin supplementation. Abbreviations; S+D (sedentary + DOX), S+P (sedentary + placebo), S+NaCu+D (sedentary + Nanocurcumin + DOX), S+NaCu+P (sedentary + Nanocurcumin + placebo), T+D (Training + DOX), T+P (Training + placebo), T +NaCu +D (Training + Nanocurcumin + DOX)

560 nm according to the ELISA method, described by Zolfagharzadeh and Roshan, [3] as the decrease suppression rate of nitrotetrazolium blue and for 1 unit of activity. Moreover, lipid peroxidation biomarker; malondyaldehyde (MDA) was measured by spectrophotometric method described by Zolfagharzadeh and Roshan [3].

Statistical analysis

Statistical analysis was performed using a commercial software package (SPSS version 21.0 for Windows). Data were normally distributed after log-transformation. A one-way analysis of variance (Statistics software, Stat Soft, Inc., Tulsa, OK) was used to detect statistical differences between groups. A post-hoc test (Tukey test) was performed to determine differences in the various biomarkers between groups. Results were presented as means ± SEM (Standard error of mean) and Differences were considered statistically significant at p-value less than 0.05.

RESULTS

Changes in body weight and relative liver weight Data on body weight and liver ratios showed, there were no significant differences among all of the groups at the start of study, DOX injection and at sacrifice time (Table 1). 15 days of DOX injection led to significant decreases in body weight at sacrifice time in the S+D and S+NaCu+D groups in comparison to S+P and S+NaCu+P groups. Table 2 also presents significant decreases in body weight at sacrifice time and after DOX injection in the T+D and T +NaCu +D group in comparison to similar saline groups. There were no significant differences in Absolute liver weight and liver to body weight ratio, in the aforesaid characteristic, among all of the groups.

Changes in liver Lipid peroxidation (MDA activity)

Administration of DOX, in S+D group caused insignificant increase in MDA levels in comparison to S+P group (P= 0.56) (Table 2). Also, Nanocurcumin Consumption in S+NaCu+D group led to insignificant increase in MDA levels in comparison to S+D group (P= 0.324). An insignificant decrease in MDA levels observed after six weeks Treadmill Running in T+D group compared to S+D group (P= 0.102). on the contrary, exercise training protocol in combination with Nanocurcumin supplementation significantly decreased MDA levels in T +NaCu +D compared to S+D group (P= 0.005). But this combination (exercise training and Nanocurcumin Consumption) has no significant effect on MDA levels in comparison with these two interventions alone (P= 0.402 and P= 0.110, respectively) (Fig 2).

Changes in liver SOD activities

Administration of DOX, in S+D group caused insignificant decrease in SOD levels in comparison to S+P group (P= 0.913) (Table 2). after of the six weeks Treadmill Running, SOD levels had an insignificant increase in T+D in comparison to S+D and T+P groups (P= 1.000 and P= 1.000, respectively). Also, an insignificant increase were also observed after Nanocurcumin supplementation in S+NaCu+D group in comparison to S+D and S+NaCu+P groups (P= 1.000 and P= 0.343, respectively). Furthermore, six weeks Treadmill Running in combination with Nanocurcumin supplementation in T +NaCu +D group, led to an insignificant decrease in SOD levels in comparison to S+NaCu+D and T+D groups (P= 0.817 and P= 0.955, respectively) (Fig. 3).

Changes in liver AIF activities

Data about AIF levels shown that, there were no significant differences between the groups (Table 2). 15 days of DOX injection led to an insignificant decrease in AIF levels in comparison to S+P group (P= 1.000). Pretreatment with six weeks Treadmill Running, were also resulted in an insignificant decrease in AIF values in comparison to similar saline group (P= 1.000). Nanocurcumin supplementation also caused an insignificant decrease in AIF levels in the S+NaCu+D group in comparison to similar saline group (P= 0.908). Also, AIF values in T +NaCu +D group, after Pretreatment with Treadmill Running and 14 days Nanocurcumin supplementation, shown an insignificant increase in comparison to S+NaCu+D group and insignificant decrease in comparison to T+D group (P= 0.995 and P= 1.000, respectively) (Fig 4).

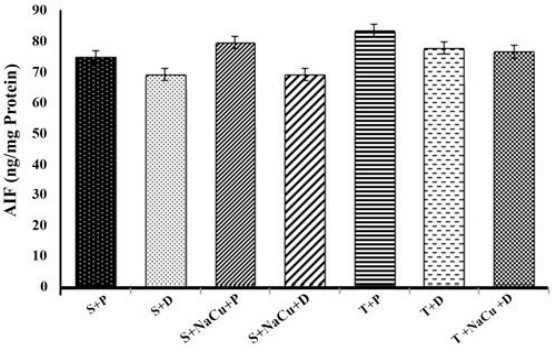

Fig 4. Apoptosis Inducing Factor (AIF) level after six weeks of Treadmill Running, DOX treatment and Nanocurcumin supplementation. Abbreviations; S+D (sedentary + DOX), S+P (sedentary + placebo), S+NaCu+D (sedentary + Nanocurcumin + DOX), S+NaCu+P (sedentary + Nanocurcumin + placebo), T+D (Training + DOX), T+P (Training + placebo), T +NaCu +D (Training + Nanocurcumin + DOX)

DISCUSSION

Our results reveal that pretreatment with six weeks Treadmill Running in combination diet supplementation with Nanocurcumin (100 mg/kg per day for 14 days) is not sufficient to protect against Dox-induced Hepatic oxidative stress and apoptosis in aging model rats.

Given that previous work has shown clinical use of Doxorubicin (DOX) as a highly effective anti-neoplastic agent, is limited by a dose-dependent toxicity in non-target tissues, including the liver [1,2,3]. During DOX therapy, the liver receives, accumulates high concentrations of DOX and it is extensively metabolized in the liver which is the cause of liver damage. Hence, it is expectable that the liver is one of the most affected organs by DOX therapy. Increasing evidence suggested the role of ROS in impairing antioxidant defense enzymes during DOX toxicity. Also, DOX-induced hepatotoxicity mainly occurs by generating oxygen free radicals, which is inhibited by free radical scavengers [5, 6, 23]. Structural injuries to the liver tissue, such as central vein congestion, inflammatory cell infiltration, hepatocellular degeneration, necrosis and fibrosis were also observed [24, 25, 26]. Many hypotheses have been proposed to counteract DOX toxicity and dysfunction, such as exercise and antioxidant supplementation [3, 4, 5, 6]. In current study, for the first time, we investigated distinct and combination effect of Treadmill Running and Nanocurcumin supplementation on MDA levels as an oxidative stress marker, SOD levels as an antioxidants enzyme and AIF levels, as an apoptotic marker in aging model rats treated with DOX.

At first, we observed a significant decrease in Animal weight status at sacrifice time, after DOX injection in the DOX groups in comparison to similar saline groups. We believe that this significant change observed in the body weight is a result of DOX induced toxicity. The other data shown that, six weeks Treadmill Running led to an insignificant increase in SOD levels and insignificant decrease in MDA and AIF values. While, other studies have previously shown regular exercise training significantly limited DOX-induced oxidant/antioxidant imbalanced and regular aerobic exercise before administration of DOX may be considered as a potentially useful candidate to protect liver tissue against oxidative stress [5,6]. Indeed, physical activity and exercise can restore the antioxidant enzymes such as SOD of liver cytosol or to the free radical scavenging activity [5,6]. Several mechanisms may explain these negative observations. First, in our study we used of a cumulative dosing (daily 1 mg/kg over the course of 15 consecutive days) and the other studies used more of an acute dosing (20 mg/kg). Second, Liver tissue collection in our study has done following 48h of DOX injection; whereas, in the previous studies Liver tissue collection have done 24h after DOX injection. Also, we believe that these converse results could be linked to the effect of aging induced by D-galactose, since other studies only used of young subjects. It is demonstrated that D-galactose injection led to accelerated aging phenotypes manifested by an increased serum advanced glycation endproducts (AGEs) level, a significant decrease in motor activity, a decrease in memory latency time, and a decrease in lymphocyte mitogenesis and interleukin-2 (IL-2) production [27]. Also, accumulated evidence has shown that with the aging increasing, the anti-oxidative defense system includes enzymes such as SOD, decline during the aging process [17]. To this evidence

and our result, it seems that pretreatment with six weeks Treadmill Running can help in cellular defense mechanisms; but to achieve the desirable and clear result in aging subjects it is recommended that designing an exercise training protocol whit different intensity, duration, time and type in future studies. In the other hand, Prevention and protection from DOX-induced hepatotoxicity has been achieved with various antioxidant supplementation such as grape seed and skin extract [2], Carnitine and Carnitine plus vitamin E [3], Korean red ginseng [7], Resveratrol [28] and particularly Curcumin [10]. Curcumin has been reported to possess anti-inflammatory, hepatoprotective antiviral activities [29]. Problems and challenges of the Curcumin, is accompanied by side effects due to its nonspecific action in conjunction with poor absorption, poor bioavailability, rapid metabolism and elimination [12]. To prevail over these weak points, previous studies suggested that, Application of nanoparticles has promising results for the poor water soluble hydrophobic agents like Curcumin [12,13,14,15]. Thus, targeted drug delivery system using nanoparticles as carrier molecules can be an effective strategy in combating DOX-induced toxicity. Therefore, for the first time in the present study, we evaluated the preventive efficacy of Nanocurcumin (100 mg /kg per day for 14 days), an encapsulated agent in ameliorating the toxic effects of DOX.

Results showed an Insignificant increase in SOD and MDA levels and insignificant decrease in AIF levels after Nanocurcumin supplementation. Previous studies have shown that, Protection by Nanocurcumin is more effective as compared to bulk Curcumin. In fact, due to the presence of beta-dike tone functional group and polyphenol structure, Curcumin possesses the ability to counteract free radicals by quenching oxygen, neutralize oxidative cascade, and thereby maintains the integrity and function of membrane and inhibits peroxidation of lipids [30]. Our observations postulates that 14 days Nanocurcumin supplementation at the dose of 100 mg/kg in aging model subjects is not sufficient in enhancing the level of antioxidant enzymes and reducing oxidative stress and apoptotic signaling. for as much as the present work is the first study that, investigating the protective effect Nanocurcumin against DOX-induced hepatic damage in aging model rats, It seems that, in the future studies it is necessary to use of the different dose of Nanocurcumin. It may be led to the better result which helps in protection of DOX-induced hepatic damage by encapsulated nanoparticles.

The most important aim of the present study was investigation combination effect of Treadmill Running exercise and Nanocurcumin supplementation on Lipid peroxidation (MDA), antioxidants enzyme (SOD) and apoptotic marker (AIF) in aging model rats treated with DOX. At first, we found that the combination of our interventions significantly decreased Lipid peroxidation levels in comparison with sedentary subjects. But altogether, our results have shown that, there were no significant differences between the groups after six weeks Treadmill Running in combination with Nanocurcumin supplementation. In fact, we found no enough results regarding the effectiveness of combination of exercise and nanoparticle antioxidants in enhancing the level of antioxidant enzymes and reducing apoptotic signaling in DOX-treated rats. In fact, all these results showed that the six weeks Treadmill Running and 14 days Nanocurcumin supplementation (100 mg Nanocurcumin /kg per day) can mitigates a little the toxic side effects of DOX treatment. But it seems that, this amount of treatment is not sufficient to protect against Dox-induced Hepatotoxicity in aging model rats. However, Despite the researcher's effort for careful control on the other influencing factors, present study had some restrictions such as, the lack of subject's diet control (energy value), lack of control over the possible impact of anesthetic (ketamine and xylazine) on biochemical indices and finally loss some of the subjects during study protocol.

CONCLUSION

Therefore, the present study concludes that Treadmill Running and Nanocurcumin can help against Dox-induced Hepatotoxicity in aging model rats. But this level of interventions does not play a required role against DOX-induced hepatic damage. Also, effectiveness of combination effect of these treatments needs more researches. The observations also suggest use of different intensity, duration, time and type in exercise training protocol and different dose of Nanocurcumin in future studies to achieving novel and effective strategies to reduce toxic side effects of DOX on the liver tissue in aging subjects.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

The experimental protocol of the current study was carried out according to the rules governing the use of laboratory animals as acceptable internationally and the experimental protocol was approved by department of sport physiology, university of Mazandaran (Registration Number: 1238304).

INFORMED CONSENT

For this type of study informed consent is not required.

REFERENCES

1.Marques-Aleixo I, Santos-Alves E, Balça MM, Moreira PI, Oliveira PJ, Magalhães J, Ascensão A. Physical exercise mitigates doxorubicin-induced brain cortex and cerebellum mitochondrial alterations and cellular quality control signaling. Mito. 2016; 26: 43-57.

2.Mokni M, Hamlaoui S, Kadri S, Limam F, Amri M, Marzouki L, Aouani E. Efficacy of grape seed and skin extract against doxorubicin-induced oxidative stress in rat liver. Pak J Pharm Sci. 2015; 1(28): 1971-1980.

3.Kumral A, Giriş M, Soluk-Tekkeşin M, Olgaç V, Doğru-Abbasoğlu S, Türkoğlu Ü, Uysal M. Beneficial effects of carnosine and carnosine plus vitamin E treatments on doxorubicin-induced oxidative stress and cardiac, hepatic, and renal toxicity in rats. Hum Exp Toxicol. 2016; 35(6): 635-643.

4.Ascensão A, Lumini-Oliveira J, Machado NG, Ferreira RM, Gonçalves IO, Moreira AC, Marques F, Sardão VA, Oliveira PJ, Magalhães J. Acute exercise protects against calcium-induced cardiac mitochondrial permeability transition pore opening in doxorubicin-treated rats. Clin Sci (Lond). 2011; 120(1): 37-49.

5.Zolfagharzadeh F and Roshan V. D. Pretreatment Hepatoprotective Effect of Regular Aerobic Training against Hepatic Toxicity Induced by Doxorubicin in Rats. Asian Pacifi J Cancer Prev. 2013; 14: 2931-2936.

6.Alishahi A, Roshan V.D, Hedayati M. Pretreatment Effects of Regular Aerobic Training on the IGF System and Hepatotoxicity Induced by Doxorubicin in Rats. Asian. Pac. J. Cancer. Prev. 2013; 14: 7427-7431.

7.Kopalli SR, Won YJ, Hwang SY, Cha KM, Kim SY, Han CK, Lee SH, Hong JY, Kim SK. Korean Red Ginseng protects against doxorubicin-induced testicular damage: an experimental study in rats. J Funct Foods. 2016; 20: 96-107.

8.Yazdanshenas A and Roshan V.D. Treadmill Running and Antioxidants Supplementation Increases Cardiac Apelin and Oxidative Stress-related Biomarkers in Rats. IJHPA. 2014; 5: 1-8.

9.He Y, Yue Y, Zheng X, Zhang K, Chen S, Du Z. Curcumin, inflammation, and chronic diseases: how are they linked?. Molecules. 2015; 20(5): 9183-9213.

10.Mohamad RH, El-Bastawesy AM, Zekry ZK, Al-Mehdar HA, Al-said MG, Aly SS, Sharawy SM, El-Merzabani MM. The role of Curcuma longa against doxorubicin (adriamycin)-induced toxicity in rats. J Med Food. 2009; 12(2): 394-402.

11.Imbaby S, Ewais M, Essawy S, Farag N. Cardioprotective effects of curcumin and nebivolol against doxorubicin-induced cardiac toxicity in rats. Hum Exp Toxicol. 2014; 33: 800-813.

12.Alizadeh AM, Sadeghizadeh M, Najafi F, Ardestani SK, Erfani-Moghadam V, Khaniki M, Rezaei A, Zamani M, Khodayari S, Khodayari H, Mohagheghi MA. Encapsulation of curcumin in diblock copolymer micelles for cancer therapy. Biomed Res Int. 2015: 824746.

13.Rahimi HR, Nedaeinia R, Sepehri Shamloo A, Nikdoust S, Kazemi Oskuee R. Novel delivery system for natural products: Nano-curcumin formulations. Avicenna J Phytomed. 2015; 17: 1-5.

14.Rachmawati H, Shaal LA, Müller RH, Keck CM. Development of curcumin nanocrystal: physical aspects. J Pharm Sci. 2013; 102: 204-214.

15.Dehghan Esmatabadi MJ, Sarkandi M, Motaleb Zadeh H, Khaledi G, Montazeri M, Zahed Shekarabi HS, Ayoubi Hormoz Y, Ali Asgari E. Comparative evaluation of curcumin and curcumin loaded-dendrosome nanoparticle effects on the viability of SW480 colon carcinoma and Huh7 hepatoma cells. Res J Pharm. 2015; 2(3): 9-16.

16.Li D, de Glas NA, Hurria A. Cancer and Aging: General Principles, Biology, and Geriatric Assessment. Clin Geriatr Med. 2016; 32: 1-5.

17.Fu C, Wang T, Wang Y, Chen X, Jiao J, Ma F, Zhong M, Bi K. Metabonomics study of the protective effects of green tea polyphenols on aging rats induced by d-galactose. J Pharm Biomed Anal. 2011;55(5):1067-1074.

18.Umanskaya A, Santulli G, Xie W, Andersson DC, Reiken SR and Marks AR. Genetically enhancing mitochondrial antioxidant activity improves muscle function in aging. Proc Natl Acad Sci U S A. 2014; 111: 15250-15255.

19.Behnke BJ, Ramsey MW, Stabley JN, Dominguez JM, Davis RT, McCullough DJ, Muller-Delp JM, Delp MD. Effects of aging and exercise training on skeletal muscle blood flow and resistance artery morphology. J Appl Physiol. 2012; 113: 1699-1708.

20.Lee J, Cho JY, Kim WK. Anti-inflammation effect of Exercise and Korean red ginseng in aging model rats with diet-induced atherosclerosis. Nutr. Res. Pract. 2014; 8(3): 284-291.

21.El-Moselhy MA, El-Sheikh AA. Protective mechanisms of atorvastatin against doxorubicin-induced hepato-renal toxicity. Biomed Pharmacother. 2014; 68(1):101-110.

22.Bajt ML, Ramachandran A, Yan HM, Lebofsky M, Farhood A, Lemasters JJ, Jaeschke H. Apoptosis-inducing factor modulates mitochondrial oxidant stress in acetaminophen hepatotoxicity. Toxicol Sci. 2011; 122(2): 598-605.

23.Carvalho C, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, Moreira PI. Doxorubicin: the good, the bad and the ugly effect. Curr Med Chem. 2009; 16(25): 3267-3285.

24.Zhao X, Zhang J, Tong N, Chen Y, Luo Y. Protective effects of Berberine on doxorubicin-induced hepatotoxicity in mice. Biol Pharm Bull. 2012; 35: 796- 800.

25.Ibrahim SS, Barakat MA, Helmy HM, Azizi J, Taimoori SR, Papzan A, SadeghAllahyari M, Morad H, Elbadr AM, Elmatary AM, Saif NA. Role of selenium in attenuating cardiac and hepatic damages induced by the antitumor agent, doxorubicin. Life Sci J. 2011; 8: 1-2.

26.Ibrahim SS, Barakat MA, Helmy HS. Modulating effect of carvedilol on doxorubicin-induced cardiomyopathy and hepatic damage. J Am Sci. 2010; 6(12): 20-32.

27.Gen-Xiang MA, Hong-Bin DE, Long-Guo YU, Dian-Dong LI, Li YY, Zhen WA. Protective role of salidroside against aging in a mouse model induced by D-galactose. Biomed Environ Sci. 2010; 23(2): 161-166.

28.Sin TK, Tam BT, Yung BY, Yip SP, Chan LW, Wong CS, Ying M, Rudd JA, Siu PM. Resveratrol protects against doxorubicin-induced cardiotoxicity in aged hearts through the SIRT1-USP7 axis. J Physiol. 2015; 593(8): 1887-1899.

29.El-Sayed EM, El-azeem AS, Afify AA, Shabana MH, Ahmed HH. Cardioprotective effects of Curcuma longa L. extracts against doxorubicin-induced cardiotoxicity in rats. J Med Plants Res. 2011; 5: 4049-4058.

30.Flora G, Gupta D, Tiwari A. Preventive efficacy of bulk and Nanocurcumin against lead-induced oxidative stress in mice. Biol Trace Elem Res. 2013; 152: 31-40.