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Original Article

The effects of advanced age on electrocardiographic parameters, lipid profile and redox balance in male rats: role of long-term cinnamon consumption

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Abstract

Background: Oxidative stress damages biological molecules and plays a role in aging-related cardiovascular diseases. *Cinnamomum zeylanicum* is a major source of antioxidants that may work against age-related cardiovascular changes. **Aims:** This study aimed to assess the changes in electrocardiography and lipid profile as well as indicators of the oxidant-antioxidant system with advanced age in rats. Also, the possible beneficial effect of cinnamon on these parameters has been investigated. **Methods:** In this experimental study, thirty male Sprague-Dawley rats were randomly assigned to five groups. The control groups of 12, and 20 months received a normal chow diet, while the investigation group was given a control diet mixed with cinnamon powder (1% of the diet). Also, a control group of 3-month-old rats was considered for assessment of age effects. Systolic blood pressure (SBP), ECG, serum lipid profiles, and heart oxidative stress markers were compared in different groups at the end of the study. **Results:** Systolic BP, serum cholesterol, HDL and LDL-cholesterol, the heart level of malondialdehyde (MDA) and nitric oxide metabolites (NOx), the ECG parameters including QRS duration, PR, T_{peak}-T_{end}, and QT interval, as well as QTc increased significantly in older rats (20 months vs 3 months). Cinnamon consumption restored MDA levels, QRS duration, and T_{peak}-T_{end} interval in aging hearts. Whereas, neither aging nor cinnamon consumption could affect SOD activity. **Conclusion:** The results demonstrated that aging is associated with ECG alteration, oxidative stress, and an increase in TC, LDL, and HDL cholesterol. Cinnamon improved electrical heart activity in aged rats (20 months) by restoration of QRS duration and T_{peak}-T_{end} interval as well as amelioration of heart oxidative stress. Altogether, cinnamon supplementation has a cardioprotective effects during aging in rats.

Key words: Aging, *Cinnamomum zeylanicum*, Electrocardiography, Heart, Oxidative stress

Introduction

The average life expectancy in the world's population is rising steadily. The significant increase of older people in societies is a challenge for medicine and biology (Collaborators, 2022; Grinin *et al*., 2023). The populations in industrialized communities are exposed to a progressive aging process that raises cardiovascular risk in societies (Aguilar-Alonso *et al*., 2018). It has been estimated that by 2030, one-fifth of the world's population will be above 65 years and cardiovascular disease (CVD) rates will increase exponentially (Izzo *et al*., 2021). Intrinsic cardiac aging is defined as the age-related degeneration and cardiac function decline that makes the heart vulnerable to stress and increases mortality and morbidity in the elderly (Wu *et al*., 2014).

Several theories are suggested to describe the "aging process"; however, the determining factors of this complex process are not yet well understood. The effect of oxidative stress over time is one of the most accepted theories. In particular, the heart is one of the organs that is affected by oxidative stress (El-Sawalhi *et al*., 2013; Marques *et al*., 2015; Martín-Fernández and Gredilla, 2016) since it is a tissue with high oxygen consumption, long cellular life, and slow turnover of antioxidant molecules (Marques *et al*., 2015). Understanding the cardiac changes that happen during the aging process is crucial, since age is a significant risk factor for

cardiovascular disease (CVD) (Pagan *et al*., 2022). Recent advancements in cardiovascular research have revealed that increased oxidative stress plays a significant role in the pathophysiological mechanisms underlying the development and progression of cardiac alterations during aging (Triposkiadis *et al*., 2019). Indeed, there is an oxidant and antioxidant imbalance associated with aging, which leads to cardiovascular damage (Wu *et al*., 2014). Studies have demonstrated an increase in lipid peroxidation in the hearts of 15-month-old rats (El-Sawalhi *et al*., 2013) and a significant increase in oxidative DNA damage in cardiomyocytes isolated from old male Fischer 344 rats (Suh *et al*., 2001). Also, many studies have shown that abnormal levels of lipids and lipoproteins in the blood are among the key risk factors for CVDs (Badalzadeh *et al*., 2014). Dyslipidemia and hypercholesterolemia lead to endothelial dysfunctions, decreased nitric oxide generation and increased production of reactive oxygen species (ROS) (Badalzadeh *et al*., 2014).

It is proposed that diets supplemented with antioxidants and the consumption of medicinal herbs can reduce ROS-induced heart damage in humans and animals (Badalzadeh *et al*., 2014; Bhupathiraju and Tucker, 2011). Some herbs are powerful antioxidants due to their polyphenolic compounds (Badalzadeh *et al*., 2014).

Cinnamon (*Cinnamomum zeylanicum*, from the Lauraceae family) which is widely used as a condiment in foods is rich in polyphenols and flavonoids (Ataie *et al*., 2019). Recently, the food and supplement industry has introduced cinnamon not only as a spice for flavoring food but also as an antioxidant and anti-inflammatory factor (Pender *et al*., 2018). It has been reported that this plant also has positive effects on the metabolism of lipids (Lee *et al*., 2003). Badalzadeh *et al*. (2014) demonstrated that long-term cinnamon supplementation improves cardiac hemodynamics in rats. They linked these effects to the reduction of MDA levels and positive changes of lipid profile (Badalzadeh *et al*., 2014).

As mentioned above, in the last century, lifespan is expected to keep rising, but knowledge of physiological aging-related processes is limited. Although global interest in medicinal herbs is growing, there is still a knowledge gap to support the beneficial effects of cinnamon as a popular spice with potential health benefits on cardiac function. Assessment of its effectiveness and underlying possible mechanisms needs further investigations. Altogether, the present study aimed to determine the changes in electrocardiography and lipid profile as well as indicators of the oxidant-antioxidant system with advanced age in rats. Moreover; the possible beneficial effect of cinnamon on these parameters has been investigated.

Materials and Methods

Ethics statement

All procedures and treatments followed international rules for laboratory animals' care. Furthermore, the study was approved by the Ethics Committee of Birjand University of Medical Sciences, Birjand, Iran (No.: IR.BUMS.REC. 1399.447, 1/4/2021).

Animals and diet

Thirty male Sprague-Dawley rats (10 weeks of age) were provided by the Research Centre of Experimental Medicine, Birjand University of Medical Sciences, Iran. All experiments were authorized by the institutional research review, and the local ethics committee approved the study (No.: Ir.bums.rec. 1399.447). The animals were housed (n=3 per cage) at an ambient temperature of 24°C with normal humidity (45-55%) and a circadian condition of a 12-hour light-12-hour dark cycle starting at 0700, which was maintained throughout the study period. The calculation of sample size is based on the "resource equation approach" design for animal studies using the formula:

$$N=[(DF/k)+1]\times k$$

Where,
N: Total number of animals
k: Number of groups
20: Is DF (Arifin and Zahiruddin, 2017)

Due to considering the possibility of mortality in animals during the study period, 6 rats were considered in each group. Rats previously used in other experiments were not included. Exclusion criteria were considered death during the experiment and animals with abnormal behavior (n=0). All efforts were made to reduce sample size and minimize animal suffering.

In the starting point of the study the animals were weighed, and then randomly divided into 5 equal groups including:

1. Control 3 months (young) (C3M): The animals received a normal chow diet until 3 months of age.
2. Control 12 months (middle-aged) (C12M): Received a normal chow diet until 12 months of age.
3. Cinnamon 12 months (middle-aged with cinnamon supplementation) (Cin12M): Received a normal chow diet until 3 months old and then consumed a normal chow diet mixed with cinnamon (1% w/w) for the next 9 months.
4. Control 20 months (old age) (C20M): Received a normal chow diet until 20 months of age.
5. Cinnamon 20 months (old age with cinnamon supplementation) (Cin20M): Received a normal chow diet until 3 months old and then consumed a normal chow diet mixed with cinnamon (1% w/w) for the next 17 months.

Electrocardiogram (ECG) monitoring, BP recording, blood, and tissue sampling were performed at the end of the study. The flow diagram of the study design is presented in Fig. 1. The experimenter who carried out statistical analysis and outcome assessment was blinded to the group allocation. The cinnamon was purchased from local store at Birjand city as Ceylon cinnamon. To confirm the constituents of the Ceylon cinnamon, the cinnamaldehyde (CNMA) was assayed by gas

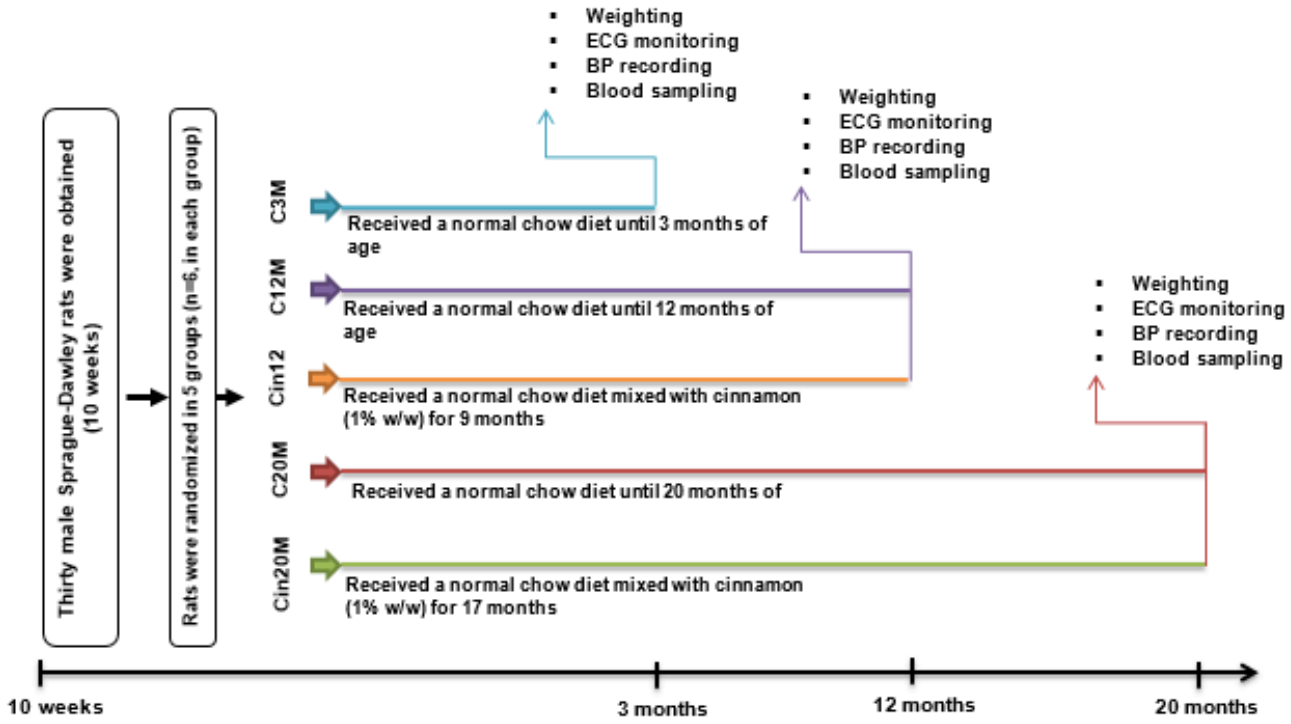

Fig. 1: Flow diagram of different experiments and the study process

chromatography, phenol and flavonoid content were measured by spectrophotometric methods (total phenol: 60.67±9.77 mg/g, total flavonoid: 121.72±8.64 mg/g and CNMA: 85%). The cinnamon dose was chosen concerning the maximum allowable dose of cinnamon in humans (Khan *et al*., 2003) and the maximum cinnamon dose determined for rats (Reagan-Shaw *et al*., 2008). The cinnamon 1% food was prepared at once by Javaneh Khorasan Co. (Mashad, Iran).

Also, the CNMA content of the cinnamon-enriched diet was measured by HPLC analysis in Professional Analysis Center and Processing of Medicinal Plants of Medicinal Plants Research Center, Institute of Medicinal Plants, Karaj, Iran (Archer, 1998). The CNMA content was 0.02 mg/g diet.

Experimental procedures

Systolic blood pressure was measured indirectly using a Piezo-Electric Pulse Transducer (AD Instruments, Australia) and an inflatable tail-cuff connected to a transducer recording pressure and PowerLab data acquisition unit (AD Instruments) in animals at the end of the study. The rats were placed in a restrainer for at least 15 min for three consecutive days to acclimate. Subsequently, SBP was measured by the tail-cuff plethysmographic method in trained conscious animals while the controller heating pad (30-32°C) warmed up small restraining cages to increase tail blood flow to improve pulse detection. After that, a cuff was placed around the proximal end of the tail of the animals. Measurements were performed after stabilization and repeated three times with 5 min intervals. The average of 3 serial measurements was considered as the SBP (Lorenz, 2002). All BP measurements were performed during 8-10 am.

Electrocardiograms were recorded in all experimental groups using PowerLab (AD Instruments, Australia) according to the manufacturer's instructions. The ECG recording was performed under pentobarbital anesthesia (Sigma, 60 mg/kg, IP). The forelimbs and right hind limb were shaved and covered with gel for attachment of an alligator clip. The apex/base recording was considered as lead II of ECG. All ECG recordings took 1 min after 5-7 min maintenance for stabilization, between 10:00 and 12:00 a.m. The following ECG parameters were examined manually in a one-minute ECG trace: QT interval (cardiac cycle duration, the interval from the onset of QRS complex to the end of the T wave), PR interval (the interval from the beginning of the P wave to the beginning of the QRS complex), T_{peak}-T_{end} interval (the interval between the peak and the end of the T wave), and QRS complex duration. All intervals are shown in Fig. 2c. Measured QT was rectified for HR using standard Bazett's formula and adjusted Bazett's equation for HR:

$$QTc\text{-}B=QT/(RR)^{1/2}$$

$$QTcn\text{-}B=QT/(RR/f)^{1/2}$$

Where,

f: The normalization factor based on the average cardiac cycle length for rats with the value of 150 ms (Kmecova and Klimas, 2010)

Heart rate was evaluated by ECG cycle length (RR duration) in six sequential beats. The ECG parameters were obtained by averaging six consecutive cardiac cycles. After recordings, animals were sacrificed using IP injection of 200 mg/kg of sodium pentobarbital, the blood sampling was performed from the heart and the

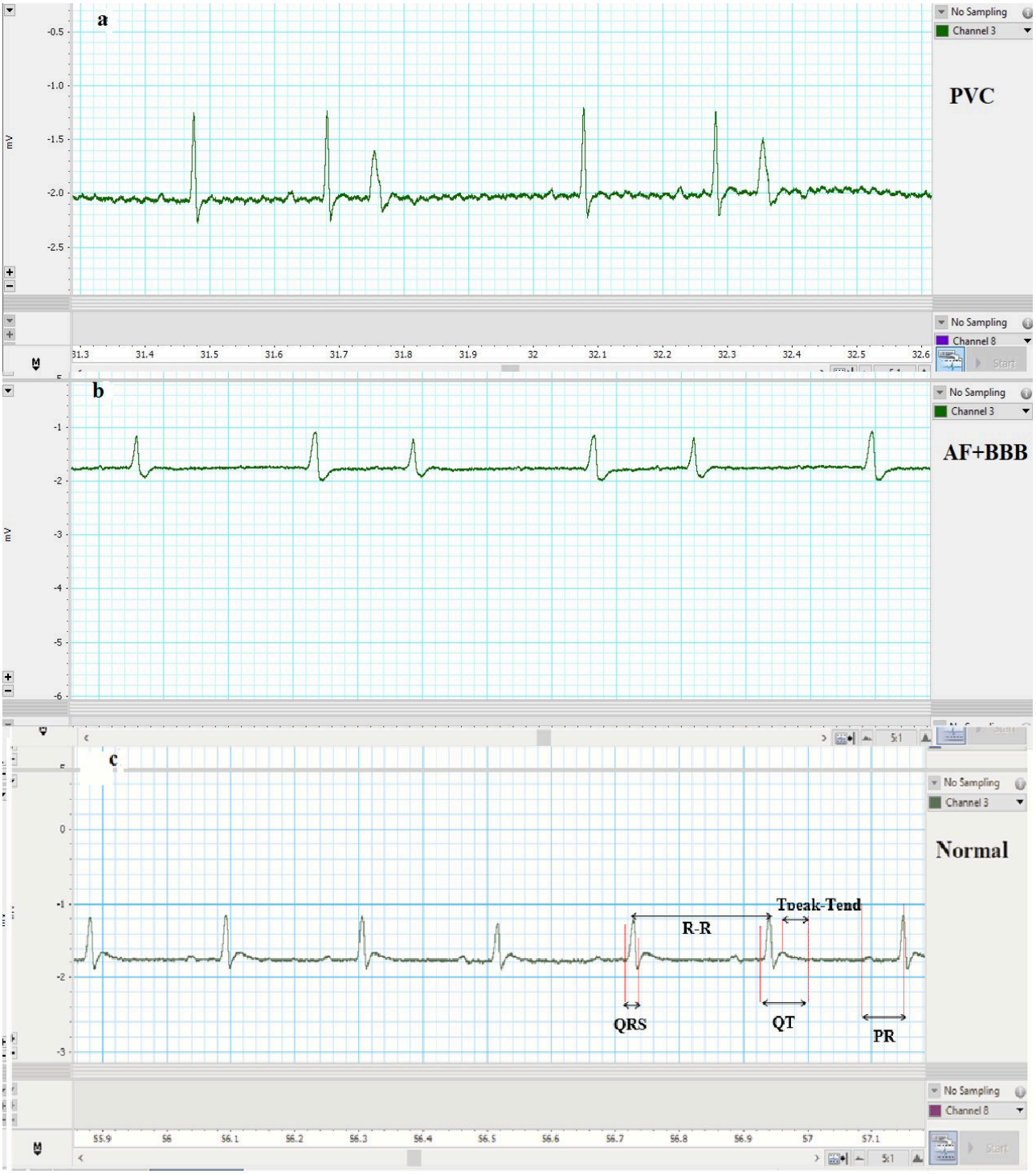

Fig. 2: The trace of ECG recording of 20-month-old animals. The ECG recordings from 20-month-old cinnamon-treated rats associated with arrhythmia of premature ventricular contraction (PVC). (**a**: Top panel) and atrial fibrillation (AF) in bundle branch block (BBB) context (**b**: Middle panel) compared to normal recording from 20-month control animals (**c**: Bottom panel). The measured intervals were shown in the normal panel. The ECG was recorded under anesthesia with pentobarbital (60 mg/kg) for 30 s

wet weight of the heart was determined. The heart weight-to-body weight ratio was calculated as a percentage and expressed as the relative heart weight.

Sera were obtained by centrifugation at 2000 g for 20 min after blood coagulation. Finally serum samples were aliquoted and stored at -80°C until measurement.

Biochemical assay

NOx measurement

Heart levels of NOx were evaluated by the Griess reaction as mentioned previously (Nakhaee *et al*., 2021). In brief, tissues were homogenized in ice-cold phosphate buffer saline (pH≈7.4, 1:10) by a mechanical homogenizer (Miccra D-1, Germany) while floating in

an ice-water small beaker. Then heart homogenate was centrifuged at 10000 g for 10 min; then, the clear supernatant was deproteinized by ethanol (90%). In detail, the same volume of ethanol and supernatant was vigorously shaken for 1 min, and the mixture was centrifuged again at 15000 g for 20 min. For NOx assay, an equal volume of deproteinized heart extract (100 µL) and vanadium chloride (saturated solution 0.8% in 1 molar HCl) was poured into a microplate; then, Griess reagent (sulfanilamide solution 0.2% in HCl 5% (50 µL) and N-(1-Naphthyl) ethylenediamine Dihydrochloride solution 0.1% in distilled water (50 µL)) was added. After a 30-minute incubation at 37°C, the absorbance of light violet-colored solution formed was read at 540 nm. NOx level was calculated from the linear standard curve established by 0-80 µmol potassium nitrate.

MDA measurement

The levels of thiobarbituric acid-reactive substances, as a byproduct of lipid peroxidation, were assayed in heart samples. Tissue extraction was performed as described above. The MDA assay was accomplished similarly to the previously described method (Ataie *et al*., 2019). Accordingly, thiobarbituric acid (0.67%, twice the sample volume) was added to a sample in acidic pH (phosphoric acid 1%, six times the sample volume) and incubated at 90-100°C bath for 45 min. Following cooling, n-butanol eight times the sample volume was added and vigorously vortexed. Then it was centrifuged at 5000 g for 10 min. The absorbance of the resulting pink product (upper phase of the final mixture) was measured spectrophotometrically at 535 nm. The heart MDA level was obtained from the linear standard curve created by 0-40 µmol 1,1,3,3-Tetraethoxypropane.

Other biochemical assays

Lipid profile [triacylglycerol (TAG), total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C)] were measured using an autoanalyzer machine (Cobas Integra, Germany) and Roche diagnostic kits (Mannheim, Germany). Heart SOD activity was determined using a commercial kit (Nasdox™-SOD Non-Enzymatic kit; Code: NS-15033, Navandsalamat, Iran). Finally, enzyme activity was calculated as:

$$(U/ml) = OD\ test/OD\ control \times 200$$

Data analysis

The normality of all data was determined by the Shapiro-Wilk test performed with Prism version 9. The data are expressed as mean±SD. For the evaluation of aging effects, different variables were compared between the C3M, C12M, and C20M groups using the one-way ANOVA/Kruskal-Wallis test. The effect of cinnamon consumption on each age group (C12M vs. Cin12M or C20M vs. Cin20M) was assessed using a t-test/Mann-Whitney U test. The one-way ANOVA and Kruskal-Wallis test were followed by a Tukey and Dunn's post-hoc test, respectively. The data with skewed distribution were presented as median (interquartile range). All graphs were created using GraphPad Prism software (version 9). The p-values less than or equal to 0.05 were considered statistically significant.

Results

The effect of aging and cinnamon consumption on SBP and relative heart weight

The heart weight to body weight ratio significantly decreased ($P<0.03$) age dependently; however, cinnamon consumption did not affect the variation of the relative heart weight pattern. The average (±SD) of the relative heart weight in C3M rats was 0.39 ± 0.03%, versus 0.34 ± 0.04% and 0.335 ± 0.01% in C12M and C20M rats, respectively (Fig. 3a). The blood pressure (BP) significantly increased in C12M and C20M groups compared to C3M rats. The mean systolic BP (±SD) in 3-month-old rats was 73 ± 6, compared with 97 ± 5 and 137 ± 10 mmHg in C12M and C20M rats, respectively. This difference was statistically significant ($P<0.001$, Fig. 3b).

The effect of aging and cinnamon consumption on ECG parameters

PR, QRS, T_{peak}-T_{end}, and QT were manually calculated on the ECG trace, as shown in Figs. 2a-c. The mean/median time of PR, QT, Tpeak-Tend, QRS duration, and QTc were significantly higher in C20M compared to C3M group. The HR was significantly lower in the C20M versus the C3M group. In the case of the C12M, we observed an increased duration of the P-R interval and T_{peak}-T_{end} compared to C3M. Also, the average QRS duration dramatically increased in the C20M versus C12M group. The QRS and T_{peak}-T_{end} duration significantly decreased following long-term cinnamon intake in the Cin20M animals (Table 1).

Two rats of the C20M group had arrhythmia (Figs. 2a and b), while all of the cinnamon-treated rats had a sinusoidal regular rhythm (Figs. 2a-c). As shown in Fig. 2a, the ECG trace with a wide QRS and a complete compensatory pause indicates premature ventricular complexes (PVCs) and in Fig. 2b, the irregular wide QRS rhythm without a P wave indicates atrial fibrillation (AF) in the context of a bundle branch block (BBB).

Effect of aging and cinnamon consumption on lipid profile

The serum lipid parameters (TC, LDL-C, and HDL-C) significantly increased with advanced age, and cinnamon consumption had no effect on pattern of these changes (Table 2). The C12M group displayed higher TC than the C3M group ($P<0.05$) (Table 2). Also, In C20M animals a significant elevation of TC and HDL-cholesterol were observed compared to C3M ($P<0.01$). The LDL-cholesterol significantly increased age-dependently (C20M>C12M>C3M). Long-term cinnamon consumption had no effect on hypercholesterolemia, and high LDL-cholesterol.

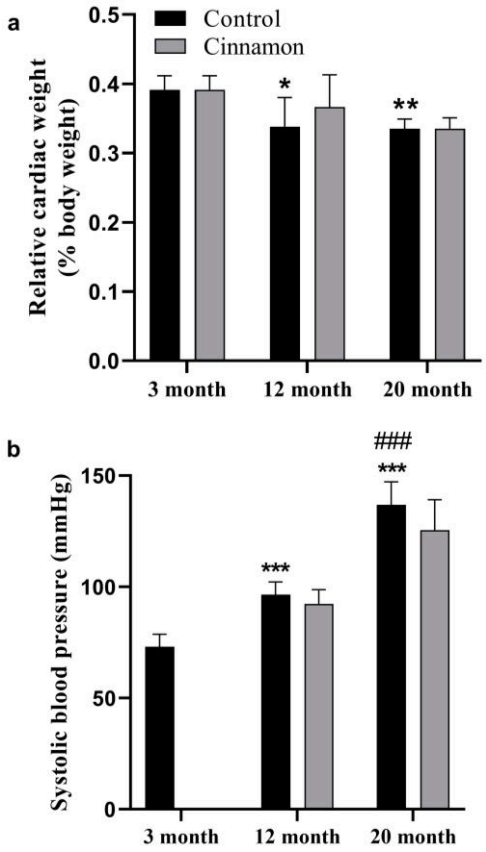

Fig. 3: The values of relative heart weight (**a**) and systolic blood pressure (**b**) in different groups. Relative heart weight was calculated as heart weight/body weight*100. The cinnamon-treated animals were fed with cinnamon 1% (w/w) from 12 weeks old until 12 and 20 months. The control groups were fed with normal diet throughout the study. The data are shown as mean±SD, and n=6 in each group. * P<0.05, ** P<0.01, and *** P<0.001 vs control 3 months group. ### P<0.001 vs control 12 months group

The effects of aging and cinnamon consumption on oxidative/nitrosative parameters

The MDA and NOx content of the heart were statistically similar in the C3M and C12M groups; however, significant elevation of these parameters was observed in the C20M animals versus the C3M and C12M groups. Cinnamon consumption ameliorated these effects and decreased heart MDA concentrations compared to the C20M group (Figs. 4a-c). Additionally, SOD activity was not affected either by cinnamon intake or with advanced age.

Discussion

Our study indicated that aging induces high blood pressure and disturbs heart electrical activity. The duration of QRS complexes and other characteristics of the ventricular action potential, such as QT or QTc time and T_{peak}-T_{end} duration, were increased in aged rats which predisposes these rats to arrhythmia. The underlying factors of heart dysfunction, such as dyslipidemia and oxidative/nitrosative stress were accelerated with senescence, which might result in functional alteration of the heart in aged rats. Widespread age-related histological changes of the conducting system, including progressive degenerative fibrosis of the AV node and the specialized His-Purkinje conduction system might also be involved in this regard (Spadaccio *et al*., 2015).

In the present study, the QRS duration was significantly longer in aged rats compared to young animals, suggesting slower intra-ventricular propagation of signals that in turn facilitates re-entry currents. Indeed, an increase in QRS duration with age has also been previously shown in humans (Macfarlane, 2018), dogs (Spasojević Kosić *et al*., 2017), and rats (Oknińska *et al*., 2021). A delay in impulse conduction that occurs in the

Table 1: Heart rate and ECG parameters in experimental groups

Groups	Heart rate (bpm)	PR interval (ms)	QT interval (ms)	Tpeak-Tend (ms)	QRS duration (ms)	QTc (ms)
C3M	329±23	40.67±5.50	58 [56, 60]	25.50 [23, 27.75]	18.33±3.14	52.37±4.08
C12M	321±3	51.00±4.63*	62 [58.50, 75]	34 [33, 35.50]*	18.40±2.07	60.61±8.48
Cin12M	317±26	51.17±4.02	62.50 [61.25, 73]	31.50 [27.75, 33]	18.67±1.36	57.84±5.73
C20M	278±26**	51.71±6.29**	78.50 [73, 85]**	45 [37.50, 50]***	28.83±9.04**, #	69.84±11.27**
Cin20M	282±45	54.33±4.76	79.50 [69.75, 80.75]	32 [30.75, 32.50]†	21.17±1.94†	63.39±7.71

* Significant versus 3-months (C3M), # Significant versus control 12-months (C12M), and † Significant versus control 20-months (C20M). * P<0.05, ** P<0.01, and *** P<0.001. bpm: Beat per minute, Cin: Cinnamon, and ms: Milliseconds. The results are mean±SD or median [interquartile range]. n=6 in each group

Table 2: Lipid profile of serum in experimental groups

Plasma parameters	Groups				
	C3M	C12M	Cin12M	C20M	Cin20M
TC (mg/dl)	53.00±3.16	73.40±12.70*	73.50±12.47	76.00±8.22**	74.00±12.70
TAG (mg/dl)	28.17±9.70	42.00±8.94	45.17±18.87	49.40±11.61	51.17±13.93
LDL-c (mg/dl)	7.00±1.26	11.20±2.28**	11.17±2.48	15.17±2.32***, ##	14.50±4.27
HDL-c (mg/dl)	33.17±2.04	41.60±8.41	51.83±7.73	43.33±6.08*	43.17±7.83

* Significant versus C3M, * P<0.05, ** P<0.01, *** P<0.001, and ## P<0.01 versus C12M. Cin: Cinnamon, M: Months, TC: Total cholesterol, TAG: Triacylglycerol, LDL-c: Low-density lipoprotein cholesterol, and HDL-c: High-density lipoprotein cholesterol. The results are presented as mean±SD. n=6 in each group

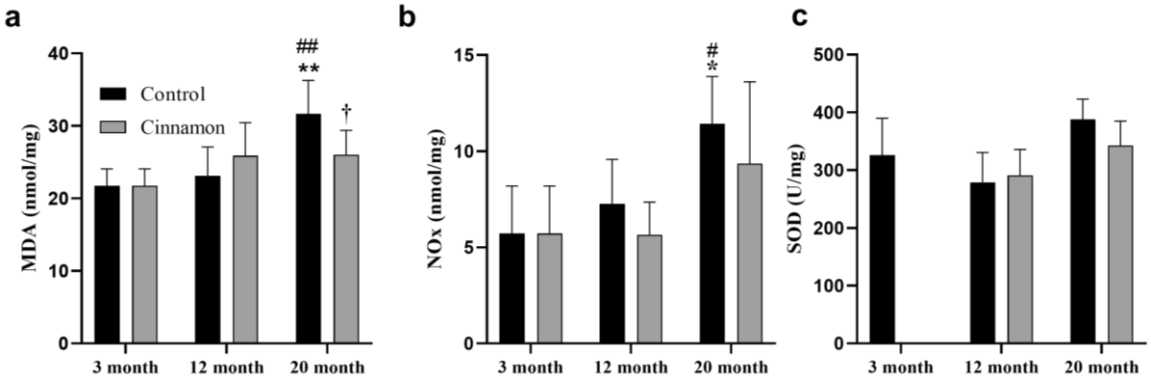

Fig. 4: The heart levels of MDA (**a**), NOx (**b**), and SOD (**c**) in different groups. The cinnamon-treated animals were fed a diet containing cinnamon 1% (w/w) from 12 weeks old until 12 and 20 months. The control groups were fed with normal diet throughout the study. The data are shown as means±SD, n=6 in each group. Significant versus control 3 months, * P<0.05, and ** P<0.01. Significant versus control 12 months # P<0.05, and ## P<0.01. Significant versus control 20 months † P<0.05. MDA: Malondialdehyde, NOx: Nitrate+nitrite, and SOD: Superoxide dismutase

bundle branches (left or right) can lead to slow intraventricular conduction, consequently wider QRS complexes may be observed without abnormal low ventricular rates (Kumar *et al*., 2012). In our study, the heart rate also decreased with aging. The lower heart rate may be associated with a higher duration of PR interval.

T_{peak}-T_{end} duration is an indicator of repolarization inconsistency. Also, it is a criterion for transmural propagation of repolarization measuring the time interval between the subepicardial (with the shortest duration of action potential) to subendocardial myocytes repolarization (with the longest action potential) (Antzelevitch and Di Diego, 2019). Overall, T_{peak}-T_{end} is a considerable point in risk assessment of arrhythmia based on repolarization heterogeneity, and a prolonged T_{peak}-T_{end} appropriately predicts arrhythmic outcome in the general population (Tse *et al*., 2017). The elongated transmural propagation (longer T_{peak}-T_{end}) is associated with more expectancy of a unidirectional conduction block and thus a re-entry current formation. Also, long QT increases the probability of early after depolarizations (EADs) (Oknińska *et al*., 2021) and consequent PVCs (as a relatively synchronized EADs progression) (Sato *et al*., 2009). This was evident in our study when a 20-month-old rat showed PVCs in ECG recording. Notably, PVCs even in the absence of apparent structural disturbances can frequently occur in the healthy elderly human population (Chadda *et al*., 2018). Another study has found age-related decline of vagal activity in 19-month-old mice that, in turn, is associated with increased vulnerability to arrhythmias (Piantoni *et al*., 2021). Collectively, age-related cardiac alterations are induced by a package of structural and electrophysiological changes.

The present findings show that aging induces oxidative stress in the heart tissue of old rats. In accordance with El-Sawalhi's study, lipid peroxidation increased in the heart of 15-month-old rats (El-Sawalhi *et al*., 2013). Cardiomyocytes isolated from old male Fischer 344 rats have shown a significant increase in oxidative DNA damage (Suh *et al*., 2001). Oxidative damage to the heart developed after 12 months of age, as MDA levels did not change in 8-month-old rats (Aguilar-Alonso *et al*., 2018). In our study, SOD activity of the heart did not change with senescence. The results of some studies have shown that SOD activity and glutathione do not increase with aging (Guo *et al*., 2009; El-Sawalhi *et al*., 2013). Another study confirmed that the SOD activity of the heart had not changed in aging rats (Aguilar-Alonso *et al*., 2018). Among the antioxidant enzymes, SOD seems to play a major role in longevity and its activity is altered by epigenetic factors (Soerensen *et al*., 2009). In our study, NOx levels increased in 20-month-old rats. With advanced age, the expression of both NOS2 (nitric oxide synthase 2) and NOS3 (nitric oxide synthase 3) increases (Zieman *et al*., 2001). In contrast to NOS1 and NOS3, which are expressed constitutively, NOS2 is induced in cells after stimulation by cytokine signals and other endotoxins or immunological agents that become abundant with aging (Barouch *et al*., 2002). NO can act either as an antioxidant or a pro-oxidant depending on its concentration. High NO production triggers apoptosis (Kim *et al*., 1999) but at the same time, it modulates transcription/translation of genes which are anti-apoptotic (Andoh *et al*., 2000). NO signaling pathways have dual effects; they can be protective or damaging depending on the context. The reduced NO bioavailability, on one hand, and atherosclerosis on the other hand, impaire endothelium-dependent vascular relaxation. Therefore, aging predisposes rats to hypertension, as documented in the present study.

In our study, the serum lipid profile adversely changed with advanced age. It seems that aging-related hypercholesterolemia is due to a lower cholesterol catabolism rate and low clearance of LDL from the circulation, (Revnic *et al*., 2019) where cholesterol absorption is unaltered (Gälman *et al*., 2007). The biosynthesis of bile acid progressively reduces with age, and then the capacity of cholesterol removal is diminished (Parini *et al*., 1999).

Long-term cinnamon consumption improved electrical activity disturbance induced by aging. The beneficial effect of cinnamon was accomplished by

inhibition of cardiac oxidative stress without restoration of age-related dyslipidemia. Few studies investigated the effect of cinnamon on arrhythmias. One study showed that oral administration of *C. zeylanicum* bark extract reduces ventricular tachycardia and ventricular ectopic beat episodes, shortens QTc, and increases heart rate during ischemia compared to the control group (Sedighi *et al*., 2018). Also, CNMA can alleviate myocardial fibrosis due to its antioxidant and anti-inflammatory effects through the involvement of many molecular pathways (Lu *et al*., 2022). On the other hand, a recent study has shown that taxifolin, an active compound of cinnamon, reveals a potential protective effect against arrhythmias in zebrafish (Xue *et al*., 2023). Thus, some constituents of cinnamon act as antiarrhythmics and may protect the heart against structural remodeling, such as fibrosis, which makes cinnamon useful for arrhythmias associated with advanced age. According to previous reports, CNMA has been shown to have hypotensive effects. A study demonstrated that CNMA can expand vascular smooth muscle of endothelium in rodents (Harada *et al*., 1982). This vasodilatory effect of CNMA may be due to its ability to inhibit both Ca^{2+} influx and Ca^{2+} release (Maruthamuthu and Ramanathan, 2016).

In accordance with the present results, cinnamon consumption has reduced the malondialdehyde (MDA) level of the heart in adult rats (Badalzadeh *et al*., 2014). In the present study, cinnamon intake overall had no effect on the lipid profile. Cinnamon consumption (1400 mg/kg/day) for 8 weeks in adult Wistar rats decreased total cholesterol (TC) and LDL-C (Badalzadeh *et al*., 2014) whereas the long-term consumption (882 days) of cinnamon extract (0.48% by weight in the diet) had no effect on TC in long-lived F1 hybrid mice (Spindler *et al*., 2013). In another study, the main component in cinnamon bark, CNMA, was administered to C57blks/j db/db mice with type 2 diabetes at a dosage of 20 mg/kg for 4 weeks. As a result, there was a decrease in triglyceride (TG) levels and an increase in HDL (Li *et al*., 2012).

The present study has a potential limitation; we could not keep animals for more than 20 months because of the high mortality rate of Sprague-Dawley rats in our lab after 20 months of age. Due to the study design - the absence of a 3-month cinnamon group - it was not possible to perform a two-way analysis of variance. Also, the electrocardiogram (ECG) and serum parameters were not investigated at baseline (before cinnamon diet feeding), so the same age control was used. Further studies are necessary to evaluate the antiarrhythmic effects of cinnamon with arrhythmia induction and to elaborate the precise antiarrhythmic mechanism of cinnamon in aged rats.

Based on the results of the present study, age-dependent alterations were observed in lipid profile and oxidative, hemodynamic, and electrocardiographic parameters of rats. Following long-term cinnamon powder consumption (1% of the diet) in aged rats (20 months), a better electrical cardiac performance - QRS duration and T_{peak}-T_{end} interval - was observed, which can be attributed to the restoration of heart MDA level while the other variables remained unchanged.

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Conflict of interest

The authors are responsible for the content of this article and confirm that there are no conflicts of interest in this publication.

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