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Ginger extract mitigates ethanol-induced changes of alpha and beta – myosin heavy chain isoforms gene expression and oxidative stress in the heart of male wistar rats

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ABSTRACT

The association between ethanol consumption and heart abnormalities, such as chamber dilation, myocyte damage, ventricular hypertrophy, and hypertension is well known. However, underlying molecular mediators involved in ethanol-induced heart abnormalities remain elusive. The aim of this study was to investigate the effect of chronic ethanol exposure on alpha and beta – myosin heavy chain (MHC) isoforms gene expression transition and oxidative stress in rats' heart. It was also planned to find out whether ginger extract mitigated the abnormalities induced by ethanol in rats' heart. Male wistar rats were divided into three groups of eight animals as follows: Control, ethanol, and ginger extract treated ethanolic (GETE) groups. After six weeks of treatment, the results revealed a significant increase in the β-MHC gene expression, 8- OHdG amount, and NADPH oxidase level. Furthermore, a significant decrease in the ratio of α-MHC/β-MHC gene expression to the amount of paraoxonase enzyme in the ethanol group compared to the control group was found. The consumption of Ginger extract along with ethanol ameliorated the changes in MHC isoforms gene expression and reduced the elevated amount of 8-OHdG and NADPH oxidase. Moreover, compared to the consumption of ethanol alone, it increased the paraoxonase level significantly.

These findings indicate that ethanol-induced heart abnormalities may in part be associated with MHC isoforms changes mediated by oxidative stress, and that these effects can be alleviated by using ginger extract as an antioxidant molecule.

1. Introduction

Experimental and epidemiological studies have demonstrated that chronic alcohol consumption is associated with cardiomyopathy and hypertension [1,2]. A wide range of alcoholic cardiomyopathy diseases, such as left ventricular dilation, left ventricular hypertrophy, myocardial infarction, and heart muscle cell proliferation has been reported by previous studies to decrease the contractility of the heart muscle and fibrosis [3]. In addition, it has been reported that chronic ethanol consumption leads to cardiac muscle cell dysfunction through abnormalities in calcium homeostasis and elevated levels of norepinephrine [4]. It has previously been shown that chronic alcohol ingestion causes a rise in blood pressure and atherosclerosis in human and animal models [4,5]. Although some features of ethanol-induced heart abnormalities have been identified by different studies, the stage intervening between the exposure of heart muscle to ethanol and initiation of the flow of responses inducing a decrease in cardiac contractility and output are still far from understood [5]. However, our recent works and various other experimental studies have provided strong evidence that oxidative stress plays a key role in ethanol negatively affecting different organs including the heart [6,2,7]. In addition, ameliorative or protective effects of antioxidant treatment during alcohol exposure support the idea that ethanol may induce its harmful effects principally through oxidative stress [6]. Oxidative nature of ethanol on the one hand, and the protective effect of antioxidant therapy, on the other hand, tempted us to design the current work with considering some precise underlying molecular mechanisms that may contribute to ethanol exerting its harmful effects on the heart during its exposure to ethanol. Therefore, based on the above mentioned observations, in this study, we focused on examining the effect of long term ethanol consumption on alpha and beta – myosin heavy chain (MHC) isoforms gene expression transition and oxidative stress in rats'

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heart. It was also planned to find out whether ginger extract mitigates the abnormality induced by ethanol in rats' heart.

2. Materials and methods

All the experiments were carried out in adherence to the Principles of Laboratory Animal Care" (NIH publication no. 85–23, revised in 1985) and were approved by the Urmia University of Medical Sciences Animal Care Committee. Twenty four male wistar rats were randomly divided into three groups: control, ethanol, and ginger extract treated ethanol (GETE) groups. Similar to our previous study, rats in the ethanol group received ethanol with a dose of 4.5 g/kg body weight (Merck KGaA, Darmstadt, Germany) saluted in tap water (20% w/v) intragastrically by gavage once a day, for six weeks.

According to previous studies, rats in the GETE group received hydro-alcoholic extract of ginger with a dose of 50 mg/kg body weight intragastrically by gavage, in addition to their regular daily diet and the same amount of ethanol [8]. The control group was treated with vehicle only (tap water).

To prepare the ginger extract, a dried ginger rhizome (originally Chinese) was purchased from a local market. Sufficient quantity of rhizome was powdered in an electric grinder. Hydro-alcoholic extract of ginger was prepared by mixing three kg of powder with six liters of ethanol 70% in a suitable container. It was then left for 72 h at the room temperature. Next, the extract was filtered through filter papers and was then concentrated using a rotary evaporator. The yield of the extract was kept in a refrigerator until the time of use [8].

The rats were anesthetized by 10% chloral hydrate (0.5 mL/kg body weight, IP), after six weeks of treatment. The depth of anaesthesia was assessed by pinching a hind paw.

At termination, after weighing of animals, the whole blood was taken directly from the heart by syringe and mixed with ethylenediamine tetraacetic acid (EDTA) as an anticoagulant. Then, the heart was removed under intrapritoneal anaesthesia with%10 chloral hydrate (0.5 mL/kg body weight). The blood sample was centrifuged at 4000 × g for 20 min within 30 min of collection. The plasma samples were stored at −80 °C without repeated freeze-thaw cycles.

The excised heart was freed from adventitial tissues, fat, and blood clots and was subsequently washed in ice-cold physiological saline and weighed. Then, the whole left ventricular wall (with septum) was excised from the heart.

For total RNA isolation, 100 mg of ventricular tissue was immersed in 1 mL RiboxEX (total RNA isolation solution) (GeneALL, Seoul, Korea) and restored at–80 °C up to the time of RNA isolation. To carry out the biochemical analysis, other parts of the ventricles were washed with ice-cold physiological saline and then dried on filter papers. Subsequently, an Ice-cold extraction buffer (10% wt/vol) containing a 50 mM phosphate buffer (pH 7.4) was added and then homogenized using Ultra Turrax (T10B, IKA, Germany). Next, the homogenates were centrifuged at 10,000*g* at 4 °C for 20 min. At the end, the supernatant sample was obtained and stored at −80 °C up to the time of analysis.

2.1. Total RNA extraction and quantitative real time −polymerase chain reaction (Real time-PCR)

The total RNA was extracted from 100 mg frozen tissue of left ventricular by using a kit (Gene all, South Korea, Cat no 305-101) according to the kit instructions. RNA concentration was verified by spectrophotometric measurement of the absorbance at 260–280 nm and determined by TAE-agarose gel electrophoresis (Fig. 1).

Reverse transcriptase (RT) was carried out using hyperscript™ Reverse Transcriptase (Gene All, South Korea). RT-PCR was performed using an amplification reagent kit (Ampliqon, Denmark) by the XP-Cycler instrument (TCXPD, Bioer, USA) with α- and β-MHC and the rats' glyceraldehydes-3-phosphate dehydrogenase (GAPDH) primers. To amplify the cDNA, the 5′ and 3′ primer sequences (forward and reverse)

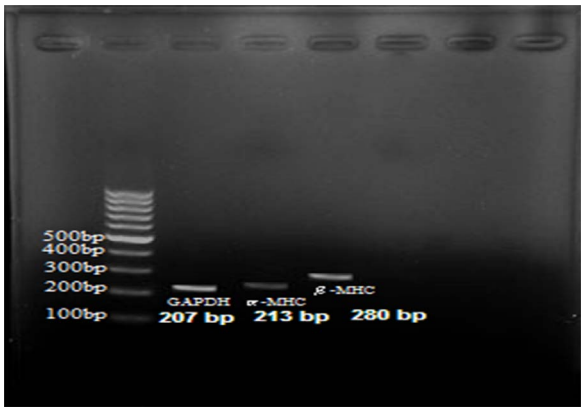

Fig. 1. Relative levels of total RNA of α-MHC, β-MHC and GAPDH in heart extracted was verified by spectrophotometric measurement of the absorbance at 260–280 nm and determined by TAE-agarose gel electrophoresis.

of the α- and β-MHC designed via the Gene Bank (http://blast.ncbi.nlm.gov/Blast.cgi) revealed that the primers were gene specific. Furthermore, Gene Runner software verified all the primers (Table 1). Primers (forward and reverse) were also synthesized to amplify the cDNA encoding GAPDH as a house keeping gene; the sequences of related primers are also provided in Table 1.

Real-time quantification of the target genes was performed by using a Real-Time PCR Master Mix Green kit (Ampliqon, Denmark) in a total volume of 25 µl and in accordance with the manufacturer's instructions. Furthermore, the mentioned genes expressions were analyzed employing an iQ5 real-Time PCR detection system (Bio-Rad, CA, USA). The reactions were then prepared for 10 min at 95 °C in a 96-well optimal plate followed by 40 cycles of 20 sec at 59 °C. In order to confirm the specificity of the amplification reactions, a melting curve was recorded. Each sample was replicated three times; the value of the threshold cycle (Ct) was the same as that of the corresponding mean. The relative expression of each mRNA was calculated by conducting the 2-ΔΔCt method, with Ct being the threshold cycle. Next, the calculated levels were normalized to GAPDH and were then analyzed for statistical significance performing a one-way analysis of variance.

2.2. Biochemical assay

2.2.1. 8-OHdG

The amount of 8-OHdG was measured by adopting the quantitative sandwich enzyme immunoassay method and using a commercial rat 8-hydroxy-desoxyguanosineElisakit (Cusabio, China). Briefly, microplates were pre-coated with a specific antibody of 8-OHdG. Then, standards and samples were pipetted into the wells, and any 8-OHdG present was bounded by an immobilized antibody. All unbound substances were removed, and avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Moreover, any remaining unbound avidin-enzyme was removed by washing. Next, a substrate solution was added to the wells and a color was developed in proportion to the amount of 8-OHdG bounded in the initial step. The color development was stopped, and the intensity of the color was measured.

2.2.2. NADPH oxidase

Assessment of the level of NADPH oxidase (NOX1) in the heart supernatant was carried out by Rat NADPH Oxidase 1(NOX1) Elisa Kit (Cusabio, China). A microplate was pre-coated with an antibody specific to NOX1. Standards and samples were pipetted into the wells and any present NOX1 was bounded by an immobilized antibody. After removing all the unbound substances, a biotin-conjugated antibody specific to NOX1 was added to the wells. Avidin conjugated Horseradish Peroxidase (HRP) was first washed and then added to the wells as well. After a wash to remove any unbound avidin-enzyme