Egypt. J. Chem. **Vol. 67,** No. 11 pp. 649 - 663 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Comparative Studies between Chinese Vs Vitnamese Panax Ginseng Exopolysaccharides and its *In Vivo* Protective Effect On Myocardial Injury Induced By Adrenaline

Tamer I. M. Ragab[a,*], Sally A. El Awdan[b], Wafaa A. Helmy[a], Abd El-Nasser G. El Gendy[c], Al Shimaa Gamal Shalaby[a]

[a]*Chemistry of Natural and Microbial Products Department, Pharmaceutical Industry Division, National Research Centre, El-Buhouth St., Dokki, Giza, 12622, Egypt*

[b]*Pharmacology Department, National Research Center, El Buhouth St., Dokki, Giza 12622, Egypt*

[c]*Medicinal and Aromatic Plants Research Department, National Research Centre, Cairo, Egypt*

Abstract

Clinically, myocardial injury (MI) is considered one of the major health problems worldwide. MI is a significant worldwide illness burden. Ginseng is a traditional medicine used in the treatment of acute decompensated heart failure (ADHF). This study aimed to assess the possible cardio protective effect of acidic, 80% methanolic (AMeOH) and 70% ethanolic (EtOH) extract from the root of Vietnamese (Vit) and Chinese (Chin) ginseng (Gens) against adrenaline-induced MI in rats. Additionally, an evaluation was conducted on the extracts' impact on histopathological alterations and cardiac function biomarkers. Adrenaline (2 mg/kg, s.c.) was given once for two days in a row (24 hours apart) in order to induce MI. Normal and control groups received the vehicle for 14 consecutive days. The other 3 groups were orally administered the extracts for 14 consecutive days. In addition, quantification of the total phenolic content as gallic acid equivalent in three extracts was measured. The antioxidant activity was measured *in vitro* using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The result revealed that acidic, 80% methanolic and 70% ethanolic extract from the root of Vietnamese (AMeOH-Vit-Gens, EtOH-Vit-Gens) and Chinese ginseng (AMeOH-Chin-Gens, EtOH- Chin-Gens) had clear cardio protective effects. Therefore, ginseng is a potential source of antioxidant specially the acidic extract and methanolic extract, respectively. The silyliated extracts were evaluated by using GC-MS analysis. The highest carboxylic acids ratio was found in the Vietnamese ginseng ethanol extract, followed by methanol extract and hydrochloric acid extract (12.17%, 10.43% and 5.64% respectively). The highest sugar content was established in Chinese ginseng hydrochloric acid extract (83.95%), Vietnamese ginseng hydrochloric acid extract (81.91%), ethanol extract (Vietnamese 79.31% and Chinese 73.03%) and methanol extract (Vietnamese 72.31% and Chinese 70.36%). Vietnamese ginseng methanol extract had 5.95% of alcohols, 2.92% of ethanol, and 3.51% of hydrochloric acid, whereas Chinese ginseng methanol extract contained 3.50% of alcohol, 5.16% of ethanol, and 0.71% of hydrochloric acid. These findings offered solid proof that ginseng might prevent myocardial injury by activating the NF-B signaling pathway.

Keywords: Myocardial injury, Vietnamese ginseng, Chinese ginseng, adrenaline-induced

1. Introduction

Across the entire world, myocardial infarction is regarded as one of the biggest health issues [1]. In the entire world, myocardial infarction is the leading cause of death [2]. When the oxygen deprivation of the heart muscle is severe enough to cause the death of myocardial cells, a heart attack occurs. The event of myocardial (heart muscle) infarction (cell death due to ischemia), which is what a heart attack is medically known as, is appropriately described by its technical nomenclature [3]. The term "myocardial infarction" refers to the death of heart cells because of an imbalance in the supply and demand of oxygen in the heart [4]. It can happen as a result of atherosclerotic plaque (type 1 myocardial infarction), changes in the oxygen supply and demand in the heart without atherosclerotic plaque (type 2 myocardial infarction), or the patient's ST elevation electrocardiogram (type 3 myocardial infarction) being detected. Types 4 and 5 myocardial infarction, respectively, can be linked to percutaneous coronary procedures or coronary artery bypass surgery [5]. In addition to being an independent condition, myocardial damage (MI) is a prerequisite for the diagnosis of MI. According to [6], oxidative stress in MI is brought on by an overabundance of reactive oxygen species (ROS), which disturbs the cardiac cell membrane and

*Corresponding author e-mail: tamerragab2006@gmail.com.;
Receive Date: 21 February 2024, Revise Date: 04 April 2024, Accept Date: 08 May 2024
DOI: 10.21608/ejchem.2024.271872.9369

causes lipid peroxidation. Elevated ROS levels in MI also cause the activation of apoptotic and inflammatory pathways. On the heart, catecholamines have both inotropic and chronotropic actions [7]. Overindulgence in catecholamines results in coronary vasoconstriction, which raises the oxygen demand and decreases the flow of blood to the heart, ultimately causing myocardial infarction. Catecholamine-induced MI is caused by an excess of reactive oxygen species (ROS) and a decrease in cardiac antioxidants, which leads to oxidative stress and myocardial necrosis or apoptosis [8].

Generally regarded as a hormone involved in the "fight or flight" response, adrenaline is a naturally occurring catecholamine [9]. The earliest application of adrenaline as a medication was in human cardiac resuscitation. It has further therapeutic uses in the management of asthma, glaucoma, allergic responses, and cardiac arrest. On the other hand, adrenaline has been shown to cause the generation of reactive oxygen species (ROS), or reactive nitrogen species-mediated tissue damages, at doses higher than physiological levels [10]. Adrenaline-induced MI in rats is considered a valid experimental model, which is used to investigate the cardio protective effect of antioxidant agents [11]. Adrenaline was found to induce MI by causing lipid peroxidation leading to depletion of cellular antioxidants [12]. In cardiomyocytes, adrenaline was shown to increase lipid, protein, and DNA damage with overproduction of nitrosative derivatives [13]. Medicinal plants and some organic products are used to cure heart attacks and have a significant positive impact on productivity [14].

Panax ginseng C. A. Meyer is a perennial plant found in Korea and northern China that is a member of the Araliaceae family. The ginseng root has been utilized for more than 2000 years, as is commonly known. A significant medicinal plant with a long history around the world is P. ginseng, sometimes known as Korean ginseng. Ginseng root is frequently employed in herbal therapy. Many studies have been conducted on the chemical components and biological functions of P. ginseng root. The P. ginseng berry exhibits possible biological and pharmacological activity, according to several studies published recently. Compared to the P. ginseng root, the P. ginseng berry has not been thoroughly investigated and analyzed. There are numerous components in ginseng's dried roots and rhizomes that are crucial for health saponins, phytosterol, carbohydrates, organic acids, nitrogenous materials, amino acids, peptides, vitamins, minerals, and some isolated and described enzymes are some of these. Ginseng saponins have been shown to be the main and most effective component among them. These saponins' extraction, purification, identification, isolation of aglycones, and biosynthesis has therefore been the subject of chemical investigation. Saponins, also known as ginsenosides or panaxosides, have been isolated and identified thus far. These triterpenes have dammarane and oleanane structures. The kind, quantity, and attachment place of the sugar moieties distinguish them from one another. These ingredients are in charge of the complicated pharmacological effects of ginseng. Moreover, it is utilized as a general tonic and adatogen to improve health by assisting the body in fending off the damaging effects of a variety of physical, chemical, and biological factors. Moreover, ginseng has powerful anti-tumor properties and can enhance immune system cell function. Although the roots of American, Japanese, San-ch'i, Himalayan, and Siberian ginseng species contain numerous saponins that are identical to those in ginseng, their general makeup is substantially different. Several of the saponins typically found in the root are present in these ginseng plants' aboveground components, particularly the leaves. Future ginseng research may focus on finding affordable supplies of ginseng saponins in nature or even through chemical synthesis. Unquestionably, more thorough, ongoing research is required to transform these traditional cures into practical, effective modern treatments [15]. Ginseng is a well-known herbal remedy that has been a crucial part of numerous Chinese treatments for thousands of years. It still has a fixed and noticeable location today. Ginseng is thought to be a valuable medicinal plant and nutritional supplement for promoting healthy biological processes, preserving human health, and regulating physiological circumstances [16]. Ginseng has recently gained popularity as a food and beverage addition as well as a dietary health supplement. These days, herbal remedies are highly valued and suggested as natural options for maintaining health. Both red and white ginsengs are effective in the treatment of cardiovascular disease [17; 18; 19]. Therefore, in this study focused on the recently reported medicinal effects of different panax ginseng species. Comparative studies between Chinese and Vietnamese panax ginseng exopolysaccharides and its in vivo protective effect on myocardial injury induced by adrenaline in rat and antioxidant, prebiotic activity was evaluated. In addition, quantification of the total phenolic content as gallic acid equivalent in acidic, methanoic and 70% ethanolic extract from the root of Vietnamese and Chinese ginseng was measured. The silyliated extracts were evaluated by using GC-MS analysis.

2. Materials and Methods

2.1. Materials

Vietnamese and Chinese Panax ginseng were obtained from the local market (Haraz Company), Cairo, Egypt. The collected ginseng plant materials

were dried in a shade at room temperature for 8 days and then milled into powder using a high-speed blender (IKA-Laboratechnic, Germany).

2.1.1 Drugs

Adrenaline 1 mg/ml S.C. ampoules (commercially available, CID, Egypt).The research protocol was in accordance and approved by Research Ethics Committee, Faculty of Pharmacy, Cairo University (REC-FOPCU).

2.1.2. Animals

Healthy adult males of Wistar Albino rats weighing 150-180g were obtained from the Animal House, National Research Centre. Rats were housed in standard polypropylene cages under standard laboratory conditions of temperature, humidity and light with a free access to standard diet and water ad libitum. The study was approved by the Institutional Medical Research Ethics Committee (MREC) of the National Research Centre (Ethical approval license Nr. 2445062023).

2.2. Methods

2.2.1. Scanning electron microscopy (SEM)

The surface morphology of the milled Vietnamese and Chinese Panax ginseng was examined using scanning electron microscopy (JEOL 5410) microscope with an accelerating voltage conducted at 10 kV. Sample were gold coated using a Hitachi coating unit IB-2 coater under a high vacuum, 0.1 Torr, high voltage, 1.2 kV and 50 mA.

2.2.2. Energy Dispersive X-Ray Spectroscopy

EDAX (or EDS) is an x-ray spectroscopic method for determining elemental compositions (qualitative and quantitative analysis).

2.2.3. Extraction and isolation

Both milled Vietnamese and Chinese Panax ginseng (50 g) was subjected to the extraction process using HCl (1N) in 1L H2O at 80°C for 3h, separately. Then supernatant was centrifuged and neutralized with NaOH (1N). After neutralization, supernatant dialyzed, concentrated and obtained acidic extract. On the other hand, the methanolic extract was obtained from the both Vietnamese and Chinese ginseng with 80% methanol at 25°C for overnight to yield a black residue. Also, ethanolic extract was obtained from both types by using 70 % ethanol at 25°C for overnight.

2.2.4. Total carbohydrate determination

Complete acid hydrolysis of extracted samples was carried out according to the modified method by [20]. Qualitative and quantitative sugars were evaluated as follow:

2.2.4.1. Qualitative examination of the hydrolyzed products

The hydrolyzolate sugars were detected using chromatography on Whatman No.1 paper, using the solvent system: n-butanol-acetone-water (4:5:1). Authentic samples of D-glactouronic acid, D-galactose, D-glucose, D-fructose, D- mannose, L-arabinoseand D-xylose were co-chromatographed as reference sugars. After chromatographic separation, the chromatogram was air dried and dipped in 40-50 ml of the color reagent, air dried, and then heated at 105°C for 10 min in an oven for developing the colored spots [21].

2.2.4.2. Quantitative Determination of the Hydrolyzed Products

Quantitative determination of the hydrolyzed sugars was done. The individual chromatographic spots were cut off, divided into small strips, and dropped into 4 ml eluting agents. The absorbance of the resulting colored solutions was determined at 390 NM Spectrophotometer UNICO 7200 [22].

2.2.5. Determination of soluble protein

Protein assay was performed according to the method of Lowry et al., (1951).

2.2.6. Total phenolic content (TPC)

The total phenolic content was estimated according to Makkar's method [23]. Three mL of the extract was thoroughly mixed with 1.5 mL of Folin Ciocalteuphenol reagent (previously diluted 1:10 in water), and allowed to stand for 5 min. Sodium carbonate solution (20%) 1.5 mL was added and the mixture was gently stirred. After incubation (90 min at room temperature), the absorbance was measured at 725 nm on a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Readings were calibrated using known concentrations of gallic acid. TPC was expressed as mg of gallic acid equivalents (mg gallic acid equivalent/ 100g extract) and the values are presented as means of triplicate.

2.2.7. Total flavonoid content (TFC)

The total flavonoid content was determined according to [24]. Briefly, 1 mL of extract was dissolved in 5 mL of H2O in a 10 mL volumetric flask. Sodium nitrite 0.3 mL (50 g L-1 in water) was added; the mixture was allowed to stand for 5 min and then 0.3 mL of aluminum chloride (100 g L-1 in water) was added. The mixture was incubated (6 min at 25°C), after that 2 mL of sodium hydroxide (1 M) was added and diluted to volume with water. The absorbance was immediately measured at 510 nm. Measurements were calibrated with a standard curve of known concentrations of catechin. TFC was expressed as mg of catechin equivalents (mg catechin equivalent/ 100g extract).

2.2.8. GC-MS analysis of silylated extracts

The Gas chromatography/mass spectrometry (GC/MS) analysis of the extracts was carried out using gas chromatography/mass spectrometry instrument stands at the department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a

TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC/MS system was equipped with a TR-5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.3 mL/min and a split ratio of 1:10 using the following temperature program: 60°C for 1 min; rising at 4.0°C/ min to 240°C and held for 1 min. The injector and detector were held at 200°C. Silylated extracts analysis was carried out as follows. Briefly,100 mg of the three fractions was extracted with 5 ml 100% methanol with sonication for 30 min with frequent shaking, followed by centrifugation at 12,000rpm for 10 min to remove debris. 100 µl of the methanolic extract was aliquoted in a screw-cap vial and left to evaporate under a nitrogen gas stream to complete dryness. For derivatization, 150µL of N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) that was previously diluted 1:1% with anhydrous pyridine was added to the dried methanolic extract and incubated at 60 °C for 45 min prior to analysis using GC-MS. Separation of silylated derivatives was accomplished on a Rtx-5MS (30m length, 0.25mm innerdiameter, and 0.25m film) [25]. Diluted samples (1:10 hexane, v/v) of 1µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70eV, using a spectral range of 40-450 m/z. The identification of the chemical constituents of the silylated polysaccharide samples was deconvoluted using AMDIS software (www.amdis.net) and identified by its retention indices (RI) with relation to n-alkanes (C6-C20). Mass spectrum matching to the Wiley spectral library collection, NSIT library database. Peaks abundance data were exported for multivariate data analysis by extraction using MET-IDEA software [26].

2.2.9. Experimental design

The rats were divided into eight groups containing six rats each. Group 1: received distilled water orally through intragastric tube daily for 14 consecutive days, then distilled water was given subcutaneously in a single dose 24 h apart fortwo consecutive days from 15th day and served as normal. Group 2: received distilled water orally through intragastric tube daily for 14 consecutive days, then adrenaline (2 mg/kg) was given subcutaneously in a single dose 24 h a part for two consecutive days from 15th day and served as control. Group 3 and 4: received orally ethanol extract Chinese and ethanol extract Vietnamese, respectively through intragastric tube daily for 14 consecutive days, then adrenaline (2 mg/kg) was given subcutaneously in a single dose 24 h apart for two consecutive days from 15th day and served as treatment. Group 5 and 6: received orally methanol extract Chinese and methanol extract Vietnamese, respectively through intragastric tube daily for 14 consecutive days, then adrenaline (2 mg/kg) was given subcutaneously in a single dose 24 h apart for two consecutive days from 15th day and served as treatment. Group 7 and 8: received orally HCl extract Chinese and Vietnamese, respectively through intragastric tube daily for 14 consecutive days, then adrenaline (2 mg/kg) were given subcutaneously in a single dose 24 h apart for two consecutive days from 15th day and served as a treatment. All the rats were sacrificed 24 h after the last dose under light anesthesia by ether. About 2 ml of blood from each rat was collected in a clean and dry test tube by cervical decapitation. The serum was separated by ultra-centrifugation (4000 rpm for 5 min) and collected by micropipette, transferred to labeled test tubes for biochemical study as follows:

2.2.9.1. AST level

AST level was estimated according to the method of [27] using rat AST ELISA assay kit. The concentration of enzyme was measured spectrophotometrically at wavelength 340 nm.

2.2.9.2. LDH level

LDH level was estimated using the enzymatic method according to manufacturer instructions (Chrono Lab, France) and measured spectrophotometrically at 340nm. Serum LDH level is expressed as U/L.

2.2.9.3. CK level

CK level was determined enzymatically using Abbot et al. (1984) method and the concentration was measured using spectrophotometer at 340 nm. Serum CK level is expressed as U/L [28].. All quantitative variables were expressed as mean ±SD. ANOVA was done for statistical analysis. Post-hocanalysis of differences was done by Least Significant Difference (LSD) test.

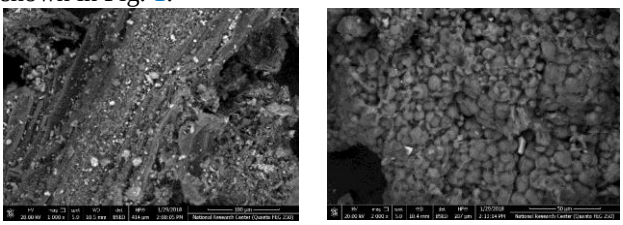
2.2.10. Histopathological examination

At the end of the experiment, animals were decapitated under light ether anesthesia. Tissue specimens were collected from the heart of the different experimental groups and preserved in 10% buffered neutral formalin (CH2O). Formalin fixed specimens were routinely dehydrated by graded series of alcohol, cleared in xylol and finally embedded in paraffin. Paraffin blocks were serially sectioned at 4-5 µm thickness and stained with H & E [29]. The obtained sections were collected on glass slides and subjected to histopathological examination using electric light microscope Olympus BH2 (Tokyo, Japan). Tissue slides were examined and compared with their corresponding controls.

3. Results and discussion:

3.2. SEM analysis

SEM spectroscopy analysis is a powerful investigative technique that uses a focused electron beam to create detailed, high magnification images of the sample surface topography. SEM indicated the presence of *Panax ginseng* Vietnamese as circle nodes; however, the Chinese has no definite shape as shown in Fig. 1.



Chinese Vietnamese

Fig. 1 Scanning electron micrograph showing the morphology and microstructure of *Panax ginseng* Vietnamese and Chinese.

3.2. EDAX analysis

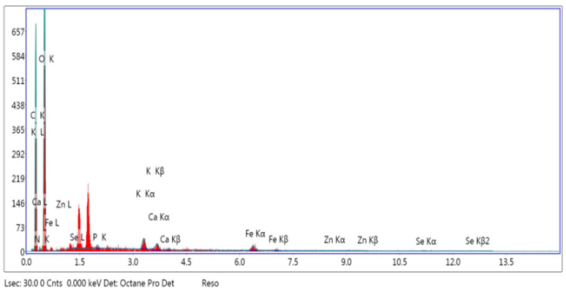
In conjunction with SEM analysis, the system's EDS component was applied to determine elements in the sample surface for qualitative and quantitative information. The ginsenosides not only make ginseng so good for our health. Also the essential minerals like (nitrogen, iron, potassium, calcium, phosphorous) and traces elements (zinc, selenium). Chinese ginseng minerals content (P 0.17%, K 0.55%, Ca 0.34% and Fe 0.63%) higher than Vietnamese ginseng (P 0.07%, K 0.38%, Ca 0.11% and Fe 0.44%). On the other side Vietnamese ginseng minerals content (N 2.89%, Zn 0.77% and Se 1.41%) higher than Chinese ginseng (N 2.34%, Zn 0.07% and Se 0.23%) as shown in Fig. 2 and Table 1. Potassium is a related mineral that is essential for maintaining a healthy heart and blood pressure. Consuming more potassium-rich foods and fewer salty foods may considerably reduce the risk of cardiovascular disease. According to research, higher serum selenium levels are linked to hypertension, although neither high nor low levels of copper or zinc are [30].

3.2. Phytochemical analysis

In the present study, we have focused on three different types of extractions from the root of Vietnamese and Chinese ginseng. One of them was using HCl (1N) at 80°C, 80% methanolic extract at 25°C and the other was an ethanolic extract. The yields of the resulted extract (Table 2) were found to comprise 1.94%, 5.20% and 4.65% of acidic methanolic and ethanolic Vietnamese ginseng extracts, respectively.

On the other hand, Chinese ginseng extracts yielded 0.53%, 5.60% and 4.57% of acidic, methanolic and ethanolic extracts, respectively.

Chinese



Vietnamese

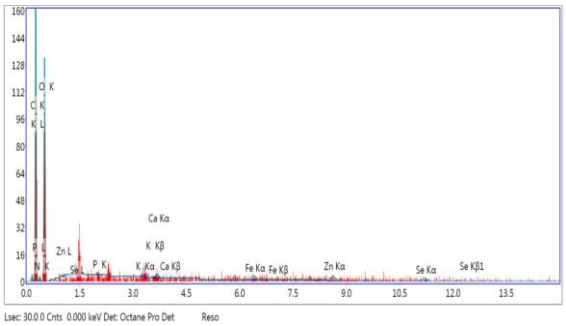


Fig. 2 EDAX analysis of Panax ginseng Vietnamese and Chinese.

Shalaby et al. (2018) reported that the yield 22% of acidic extract while, 40% was found in the alkaline Vietnamese ginseng extract. Therefore, the analytical characterization of acidic extract indicated that the presence of carbohydrates (83% Chinese ginseng extract and 81% Vietnamese ginseng extract) as major constituent. However, the methanolic and ethanolic extract has 70-72% total carbohydrates content (TCC) [31]. Total soluble protein (TSP) ranged between 1.3 to 5%. Using the Folin-Ciocalteu reagent method, the total phenolic contents (TPC) of several P. ginseng extracts were examined, and a remarkable quantity was discovered in all samples (Table 2). TPC was estimated 5.95 mg/ml methanolic, 2.92 mg/ml ethanolic and 3.51 mg/ml acidic Vietnamese ginseng extracts, on the other hand Chinese ginseng extracts recorded 3.50 mg/ml methanolic, 5.16 mg/ml ethanolic and 0.71 mg/ml acidic extract. The total flavonoid content (TFC) was determined and lowest content was 17.11 mg/ml acidic Chinese extract, 19.91 mg/ml ethanolic Vietnamese extract and the highest content was methanolic Chinese extract 28.21 mg/ml. The P. ginseng root, leaf, and fruit all contained TPC, but the methanolic fruit extract had the highest quantity (95.21 mg/g). Methanolic leaf extract (39.21 mg/g), which was followed by root (30.21 mg/g), had the second-highest concentration. Methanolic fruit extract was found to have the highest total flavonoid concentration (50.21 mg/g), followed by leaf (24.32 mg/g) and root (20.25 mg/g) [32]. It was established that the composition of the dry root was approximately as follows: ginsenosides, 1–6%;

carbohydrates, 60–70%; nitrogen-containing compounds, 12–16%; fats, 2%; vitamins, 0.05%; mineral 4–5% and moisture, 9–11% [33].

Qualitative and Quantitative carbohydrate analysis was determined and the results revealed that different monosaccharide existed. Glucose (50-80%), arabinose (10-40%), fructose (5-15%) and galactose (2.5-5%) were the main sugars. Glucouronic acid was represented as traces sugar as shown in Fig. 3.

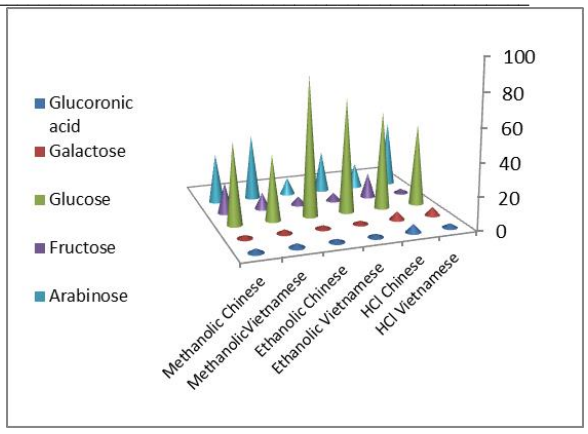


Fig. 3 Qualitative and Quantitative monosaccharide sugars analysis.

Table 1 Elemental content of Panax ginseng Vietnamese and Chinese.

Element	Chinese			Vietnamese		
	Weight %	Atomic %	Error %	Weight %	Atomic %	Error %
C	37.23	44.52	8.00	46.89	54.96	9.91
N	2.34	2.40	34.91	2.89	2.91	97.56
O	58.43	52.46	10.66	47.04	41.4	14.04
P	0.17	0.08	35.45	0.07	41.4	14.04
K	0.55	0.20	15.23	0.38	0.14	50.99
Ca	0.34	0.12	22.29	0.11	0.04	77.77
Fe	0.63	0.16	21.76	0.44	0.11	67.51
Zn	0.07	0.02	77.51	0.77	0.17	66.8
Se	0.23	0.04	73.32	1.41	0.25	69.06

Table 2 Phytochemical analysis of three different types of extractions from the root Vietnamese and Chinese ginseng.

Panax ginseng	Extract	Yield	TCC	TPC	TFC	TSP
Vietnamese	Acidic	1.94	81	3.51	21.63	3.61
	Methanolic	5.20	72	5.95	27.98	4.28
	Ethanolic	4.65	72	2.92	19.91	4.62
Chinese	Acidic	0.53	83	0.71	17.11	1.31
	Methanolic	5.60	70	3.50	28.21	5.01
	Ethanolic	4.57	70	5.16	25.77	4.66

3.4. GC-MS analysis of silylated extracts

By comparing each compound's retention time, peak area, and interpretation of its mass spectral data to the literature, all compounds were identified. Based on the information in Fig. 4 and Table 3, it was determined that 66 components were present in total, accounting for 96.61%, 99.84%, and 97.18% of the total Vietnamese ginseng extracts of methanol, ethanol, and hydrochloric acid, on the other hand 98.45%, 98.84%, and 92.13% of the total Chinese ginseng extracts of methanol, ethanol, and hydrochloric acid, respectively. The highest carboxylic acids ratio was found in the Vietnamese ginseng ethanol extract, followed by methanol extract and hydrochloric acid extract (12.17%, 10.43% and 5.64% respectively). Phosphoric acid was the main carboxylic acid (6.31%) and then Malic acid (4.31%). However, citric, quinic, malonic and malic acid were found just in Vietnamese ginseng extracts. The obtained carboxylic acids ratio was agreed with Ragab et al. 2021 (21.40%) [34]. Vietnamese ginseng methanol extract had 5.95% of alcohols, 2.92% of ethanol, and 3.51% of hydrochloric acid, whereas Chinese ginseng methanol extract contained 3.50% of alcohol, 5.16% of ethanol, and 0.71% of hydrochloric acid. Myoinositol was the highest alcohols 4.01% in Vietnamese ginseng methanol extract, 2.85% in ethanol, and 2.98% in hydrochloric acid extract. The only sources of linalool, glycerol, erythritol, and barigenol were Chinese ginseng extracts. In all extracts, amino acids were present in roughly similar amounts. Pyroglutamic acid was the most abundant amino acid, followed by alanine, valine, proline, serine, and aspartic acid. Fatty acids

represented 2.29% in Chinese ginseng ethanol extract as tetracosanoic acid, 1.86% in Chinese gensing methanol extract as tetracosanoic acid and oleanolic acid, and 1.31% in Vietnamese gensing methanol extract as oleanolic acid. Linoleic acid was only 8.17% in Vietnamese ginseng butanol fraction [34]. Octadecanamide 4.9 % was found as amide in Chinese ginseng ethanol extract, 4.46% in Chinese ginseng methanol extract and less than 1% in other extracts. Adenosine was found as the main amine in all extracts and then Phenylethanolamine. Hydroxyandrosterone and methylcytosine were existed as ketons traces (less than 1%). Amides, amines, ketons and furans were represented as traces in Vitnamese ginseng fractions [34]. Ginseng generally had many carbohydrates, which were visible in their extracts. the highest sugar content was established in Chinese ginseng hydrochloric acid extract (83.95%), Vitnamese ginseng hydrochloric acid extract (81.91%), ethanol extract (Vitnamese 79.31% and Chinese 73.03%) and methanol extract (Vitnamese 72.31% and Chinese 70.36%). Fructose was the main ketonic monosaccharide sugar in all extracts ranged from 26.79% -14.66%. Glucopyranose was the second abundant sugar in Vitnamese ginseng (20.68%- 11.66%) and galactopyranose (15.77%-8.59%), respectively. Sucrose was the disaccharide sugar existed in all extracts (22.55%-1.40%).The sugar contents of the Vitnamese ginseng were as follows: fructose 22.62%, sucrose 22.61%, glucopyranose 12.67%, and galactopyranose 10.16% [34].

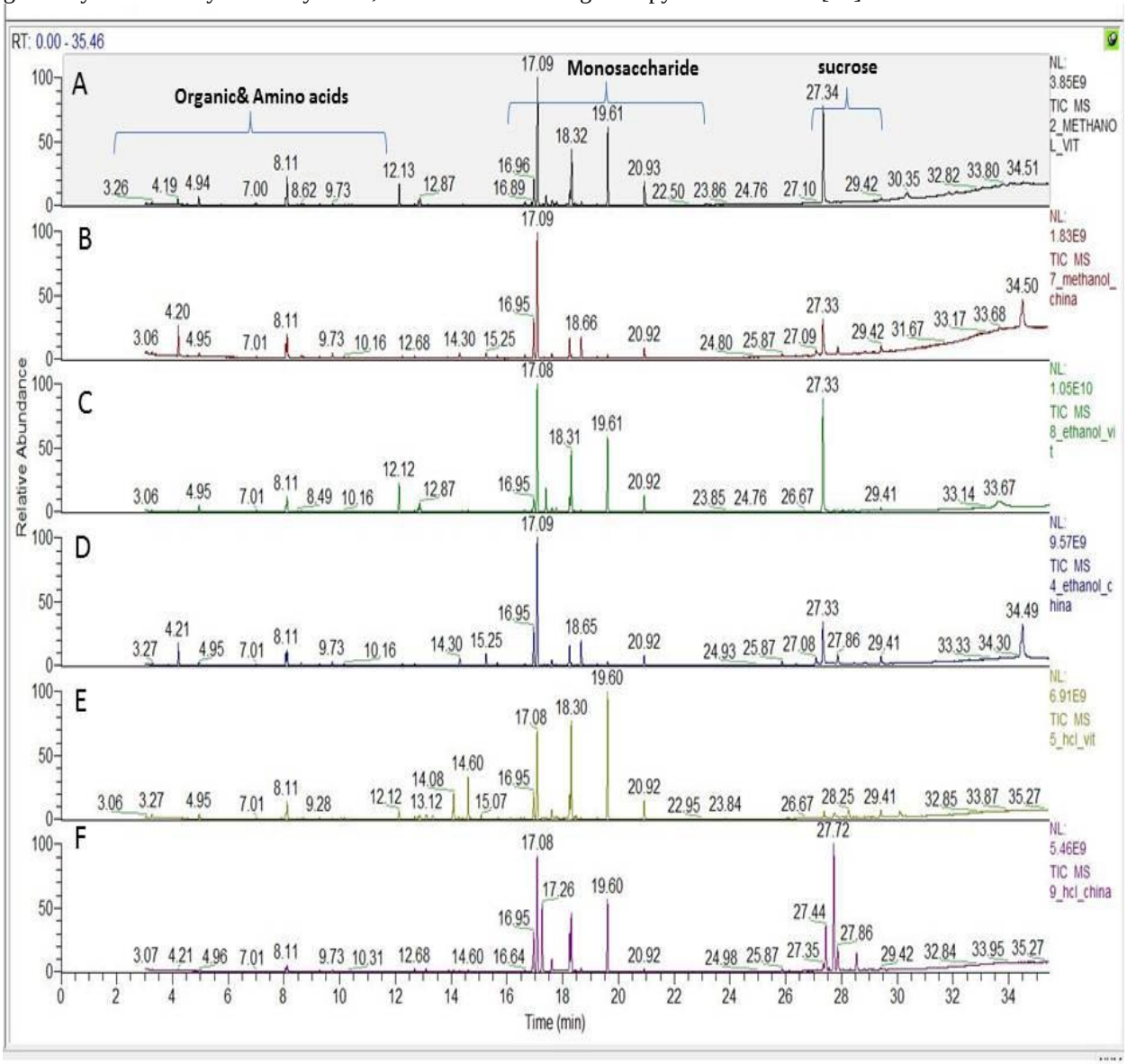


Fig. 4 GC–MS chromatogram of silylated methanol extract Vit. (A), methanol extract Chin. (B), ethanol extract Vit. (C), ethanol extract Chin. (D), HCl extract Vit. (E) and HCl extract Chin. (F).

Table 3 Chemical profiles of silylated methanol extract Vit., methanol extract Chin., ethanol extract Vit., ethanol extract Chin., HCl extract Vit. and HCl extract Chin.

				Methanol ext.		Ethanol ext.		HCl ext.		Structure
Peak	**Name**	**RT(min)**	**KI Exp**	**VIT**	**China**	**VIT**	**China**	**VIT**	**China**	
1	Propylene glycol	3.26	988	0.25	0.4	0.07	0.19	0.37	0.07	C9H24O2Si2
2	D-Lactic acid	4.19	1047	0.73	4.64	0.14	3.26	0.18	0.15	C9H22O3Si2
3	Alanine	4.94	1094	1.08	0.73	0.89	0.47	0.62	0.14	C9H23NO2Si2
4	Malonic acid	6.95	1202	---		0.1	---	---	---	C9H20O4Si2
5	L-Valine	7.00	######	0.36	0.29	0.26	0.19	0.24	---	C11H27NO2Si2
6	Linalool	7.92	1157	---	---	---	---	0.16	---	C13H26OSi
7	Glycerol	8.06	1259	---	---	---	1.94	---	---	C12H32O3Si3
8	Phosphoric acid	8.11	1263	5.02	6.31	3.16	2.47	3.36	1.16	C9H27O4PSi3
9	Isoleucine	8.49	1282	---	---	0.16	---	0.13	---	C12H29NO2Si2
10	Proline	8.62	1324	0.33	0.52	0.14	0.45	---	0.11	C11H25NO2Si2
11	11-β-Hydroxyandrosterone	8.7	1307	---	0.23	0.07	0.14	---	---	C19H30O3
12	Proline	9.28	1324	---	0.37	0.09	0.2	0.29	0.13	C17H37NO2Si2
13	Serine	9.73	1348	0.27	1.12	0.12	0.75	0.22	0.24	C12H31NO3Si3
14	L-Threonine	10.16	1371	---	0.38	0.17	0.33	0.16	0.08	C13H33NO3Si3
15	Phenylethanolamine	10.31	1379	---	0.28	0.07	0.18	0.19	0.09	C17H35NOSi3
16	Malic acid	12.13	1479	3.02	---	4.31	0.17	1.3	---	C13H30O5Si3
17	Erythritol	12.25		---	0.51	---	0.25	---	---	
18	L-Aspartic acid	12.69	1510	0.27	0.41	0.24	0.33	0.39	0.45	C13H31NO4Si3
19	γ-Aminobutyric acid	12.82	1518	0.65	---	0.62	0.12	0.38	---	C13H33NO2Si3
20	Pyroglutamic acid	12.87	1521	1.32	---	1.69	0.24	0.62		C11H23NO3Si2
21	5-Methylcytosine	13.15	1537	---	---	0.11	---	1.03	0.34	C11H23N3OSi2
22	L-Threonic acid	13.22	1542	---	---	0.1	---	---	---	C16H40O5Si4
23	Barrigenol R1	13.85	---	---	0.39	---	0.25	---	0.16	C30H50O6
24	α-DL-Arabinopyranose	14.09	1592	---	---	0.14	---	4.68	0.16	C17H42O5Si4
25	Ornithine	14.3	1603	---	1.19	---	1.47	0.37	0.16	C14H36N2O2Si
26	Glutamic acid	14.4	1613	---	---	0.24	0.11	0.32	---	C14H33NO4Si3
27	N,O-Bis-phenylalanine	14.53	1619	---	---	0.11	---	---	---	C15H27NO2Si2
28	Arabinose	14.6	1623	---	---	0.16	---	6.24	0.21	C17H42O5Si4
30	Tetracosanoic acid	15.25	---	---	1.14	---	2.29	---	---	C27H56O2Si
31	D-Xylofuranose	15.65	1689	---	0.6	0.24	0.58	0.43	---	C17H42O5Si4
32	Fructofuranoside, methyl	16.65	1750	0.71	0.59	0.46	0.37	0.4	0.19	C19H46O6Si4
33	D-(-)-Tagatofuranose	16.89	1767	0.49	---	0.25	0.14	---	---	C21H52O6Si5
34	D-(-)-Fructofuranose	16.96	1771	4.32	7.67	2.59	7.27	4.38	6.24	C21H52O6Si5
35	FRUCTOSE	17.09	1779	21.42	26.06	22.56	26.79	14.66	17.32	C21H52O6Si5
36	d-(-)-Fructose	17.32	1794	---	---	0.14	0.21	0.3	9.35	C21H52O6Si5
37	Citric acid, tetra	17.41	1800	1.71	---	3.83	0.17	0.31	---	C18H40O7Si4
38	D-Psicopyranose	17.62	1814	0.97	0.93	0.61	1.06	1.71	1.76	C21H52O6Si5
39	Quinic acid	17.78	1825	0.45	---	0.63	---	0.49	---	C22H52O6Si5
40	D-Psicose	18.26	1858	2.06	4.22	2.18	4.1	3.58	5.17	C21H52O6Si5
41	Galactopyranose	18.32	1860	8.59	---	10.13	---	15.77	7.78	C21H52O6Si5
42	α-D-(+)-Talopyranose	18.43	1869	0.23	---	0.3	---	0.35	0.14	C21H52O6Si5
43	Octadecanamide	18.66	1898	0.62	4.46	0.2	4.9	0.89	0.67	C22H44N2O2
44	Glucosamine per	19.23	1925		0.45	0.13	0.44	0.17	---	C24H61NO5Si6
45	β-D-Glucopyranose	19.61	1951	11.66	0.78	12.64	0.75	20.68	9.77	C21H52O6Si5
46	Myoinositol	20.93	2062	4.01	2.2	2.85	1.92	2.98	0.37	C24H60O6Si6
47	Glyceryl-glycoside	23.85	2351	---	---	0.11	---	---	0.39	C27H66O8Si6
48	Hexopyranose	26.67	2628	---	0.67	0.15	1.1	---	---	C21H52O6Si5
49	D-Psicofuranose	27.09	2670	---	1.78	---	1.97	---	0.3	C21H52O6Si5
50	Sucrose	27.34	2693	19.2	9.46	22.55	10.56	1.4	1.16	C36H86O11Si8
51	B-LYXOPYRANOSE	27.77	2732	---	---	---	---	1.67	19.98	C17H42O5Si4
52	Adenosine	27.86	2745	---	1.8	0.26	2.16	0.27	3.75	C22H45N5O4Si
53	Maltose	28.26	2786	---	---	0.11	---	2.19	0.45	C36H86O11Si8
54	Aucubin	28.46	2804	---	0.7	---	0.42	---	2.81	C33H70O9Si6
55	D-Lactose	28.83	2847	---	0.77	---	0.72	---	0.2	C36H86O11Si8
56	1-Monolinoleoylglycerol	29.15	2873	---	---	---	---	---	0.11	C27H54O4Si2
57	Monostearin	29.42	2899	0.74	2	0.53	1.68	1.57	0.57	C27H58O4Si2
59	β-Gentiobiose	30.09	2966	---	---	0.21	0.48	1.45	---	C36H86O11Si8
60	4-O-β-Galactopyranosyl-D-mannopyranose	30.28	2984	---	---	---	---	0.28	---	C36H86O11Si8
61	β-Sitosterol	30.35	---	2.29	---	---	0.51	---	---	C32H58OSi
62	Oleanolic acid	31.85	3140	1.31	0.72	---	---	---	---	C30H48O3
63	GLYCOCHOLIC ACID	32.12	---	0.67	0.68	---	0.28	---	---	C30H50O2
64	D-(+)-Turanose	33.68	3318	0.49	1.1	3.02	0.58	---	---	C36H86O11Si8
65	Stigmasterol	34.2	3370	0.61	---	---	0.35	---	---	C32H56OSi
66	Sucrose	34.51	---	0.76	11.9	---	13.53	---	---	C27H54O4Si2
	Total			**96.6**	**98.45**	**99.8**	**98.84**	**97.2**	**92.13**	

3.5. Chemical composition-based main component analysis (PCA) and agglomerative hierarchical clustering (AHC)

The chemical compound data were applied to PCA in a trial to analyses the variation within ginseng extracts in an untargeted manner, despite the fact that different nonvolatile patterns of several extracts of ginseng were visible by simple visual inspection of the GC-MS chromatograms (Fig. 4, Table 3). The PCA is an unsupervised clustering method that does not require data set awareness and works to keep the variation in the data while reducing the multivariate data dimension [25]. Based on the PCA, the main component correlations among the investigated ginseng extracts were examined (Fig. 5). The PCA horizontal axis explained 69.90%, while the vertical axis explained a further 15.50% of the overall variation. The findings deduced the correlations among the two ginseng locations and different ginseng extracts, and all these extracts and ginseng from two location were found on the positive side of the cluster. The methanol and ethanol fraction showed strong correlations based on the abundance of sugars and amino acids, especially the major compounds; fructose, sucrose, β-D-glucopyranose, galactopyranose, and others.

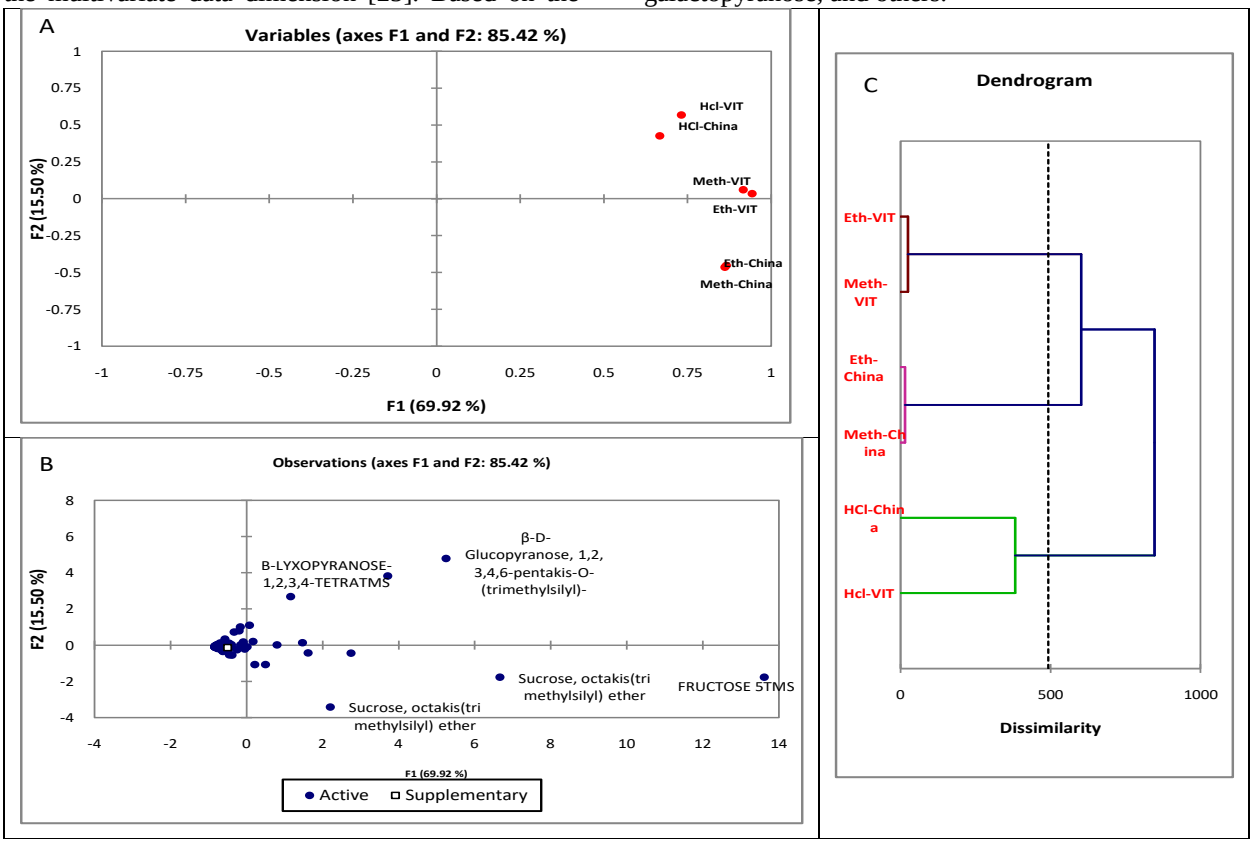
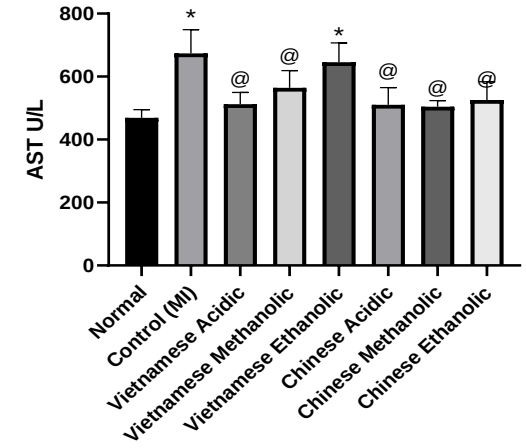


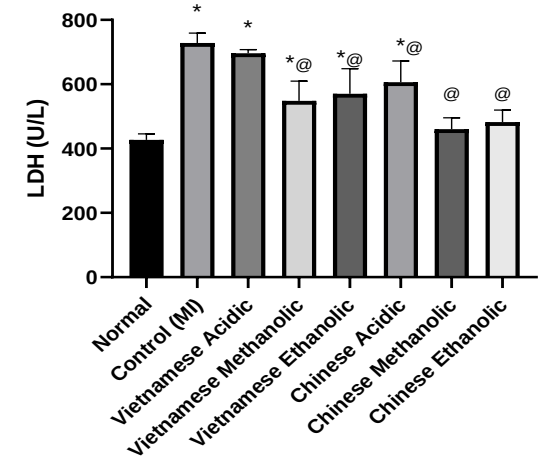
Fig. 5 Hierarchical clustering and principal component analyses of silylated metabolites from different ginseng extracts. (A) Score plot of PC1 vs. PC2 scores. (B) Loading plot for PC1 & PC2 contributing metabolites and their assignments. (C) HCA plot. The metabolome clusters are located at the distinct positions in two-dimensional space described by two vectors of principal component 1 (PC1)=69.92% and PC2=15.50%.

3.6. Effect of the extracts on serum and cardiac biochemical parameters

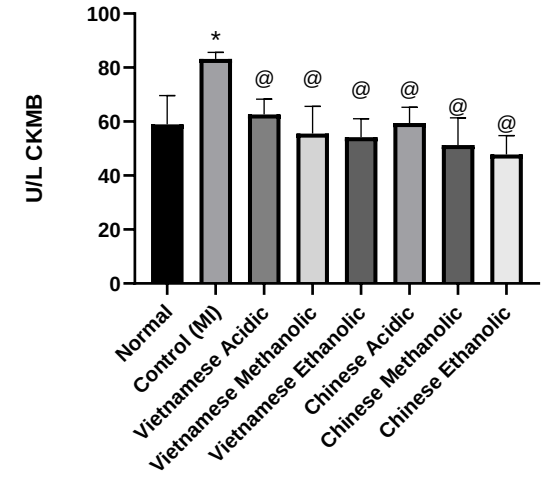
Serum and cardiac enzymes in the studied groups at the end of the experimental period, as depicted in Figures 6A, 6B and 6C adrenaline-induced MI resulted in significant increase in serum AST, LDH, and CK levels as compared to normal rats. Oral pretreatment of all extracts except Vietnamese ethanolic extract showed a significant decrease in AST as compared to control (MI) group. Oral pretreatment of all extracts except Vietnamese acidic showed a significant decrease in LDH level as compared to control (MI) group. Oral pretreatment of all extracts showed a significant decrease in CK-MB level as compared to control (MI) group.



A



B



C

Fig. 6 Effect of extracts on serum and cardiac enzymes (A) AST, (B) LDH and (C) CK-MB.

This study provides new evidence on the cardio protective effect of extracts from the root of Vietnamese and Chinese ginseng against adrenaline-induced MI in rats. To the best of the authors' knowledge, this is considered the first study to investigate the role of cardiac enzymes, as well as histopathological changes of extracts from the root of Vietnamese and Chinese ginseng in adrenaline-induced MI in rats. Subcutaneous administration of adrenaline (2 mg/kg) for 2 consecutive days in the present study resulted in abnormalities as manifested by elevated serum levels of cardiac enzymes; AST, LDH, and CK levels as described in a previous study [35]. This reveals that adrenaline administration led to myocardial damage and leakage of cardiac enzymes into the circulation. Administration of extracts from the root of Vietnamese and Chinese ginseng against adrenaline-induced MI in rats reduced serum levels of AST, CK and LDH, reflecting preservation of membrane integrity and thereby cardio protection against adrenaline-induced MI. Those findings are paralleled by improved histopathological features of cardiac lesions and are in consistence with other studies about natural products that efficiently attenuated cardiac damage in adrenaline-induced MI in rats [36].

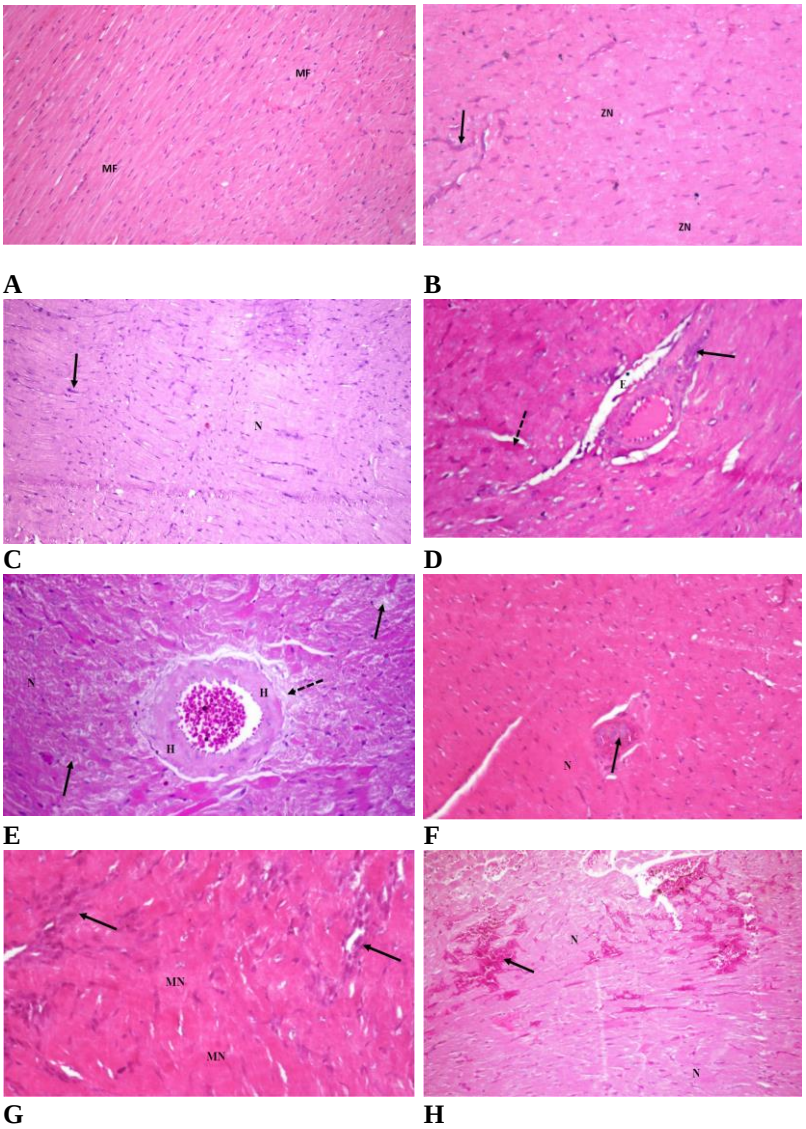
3.7. Histopathological examination

Microscopic examination of hearts of control negative rats showed normal histological structure of the cardiac muscle fibers (Fig. 7A). While the hearts of administrated rats (rats with infarcted hearts) showed widespread areas of Zenkers' necrosis of the myocardial muscle fibers and few inflammatory cell infiltrations **(**Fig. 7B). In the focal necrotic areas, the myofibrils appeared homogenous without any nuclear structure with scattered inflammatory cell infiltration (Fig. 7C). The blood vessels showed inflammatory cell infiltration in the blood vessel's wall and focal areas of hyalinization and perivascular edema (Fig. 7D) with swelling and granular degeneration as well as necrosis of the cardiac muscle fibers (Fig. 7E). Focal areas of calcification were seen in the necrotic cardiac muscles (Fig. 7F).

Regarding examination of various treated groups, revealing variable degrees of curative effects. Hearts of control positive rats (infarcted hearts) which treated with methanolic Chinese extract showed widespread myonecrosis and mononuclear inflammatory cell infiltration (Fig. 7G). Areas of inter-muscular hemorrhages accompanied a moderate degree of necrosis of the muscle fibers were also apparent (Fig. 7H). Hearts of control positive rats (infarcted heart) and treated with methanolic Vitnamese extract showed diffuse myonecrosis and foci of hyalinization (Fig. 7I). Some hearts showed a moderate degree of myonecrosis with an obvious new

vascularization among the necrotic fibers (Fig. 7J). Concerning hearts of control positive rats (infarcted heart) and treated with ethanolic Chinese extract, the examination of which revealed few mononuclear inflammatory cell infiltration, new vascularization among the necrotic muscle fibers (Fig. 7K). Generally, the moderated degree of myocardial necrosis and marked, scattered vascularization with rare intermuscular hemorrhages was a prominent finding (Fig. 7L). In regards to rats with infarcted hearts and treated with ethanolic Vitnamese extract showed a fair degree of necrosis of the myocardial muscle fibers and few extravasated blood and few vascularization (Fig. 7M and 7N). Rats with infarcted cardiac tissue and treated with HCl extract (China) showed intermuscular hemorrhages, and intermuscular edema accompanied areas of myocardial necrosis (Fig. 7O). Most of the blood vessels in those hearts showed vascular congestion with vascular wall hyalinization (Fig. 7P).

However, hearts of control positive rats (infarcted heart) and treated with HCl extract (Vitnamese) showed severe swelling and homogenization (Zenkers' necrosis) of the cardiac muscles and few fibers with cytoplasmic vacuolation (Fig. 7Q and 7R).



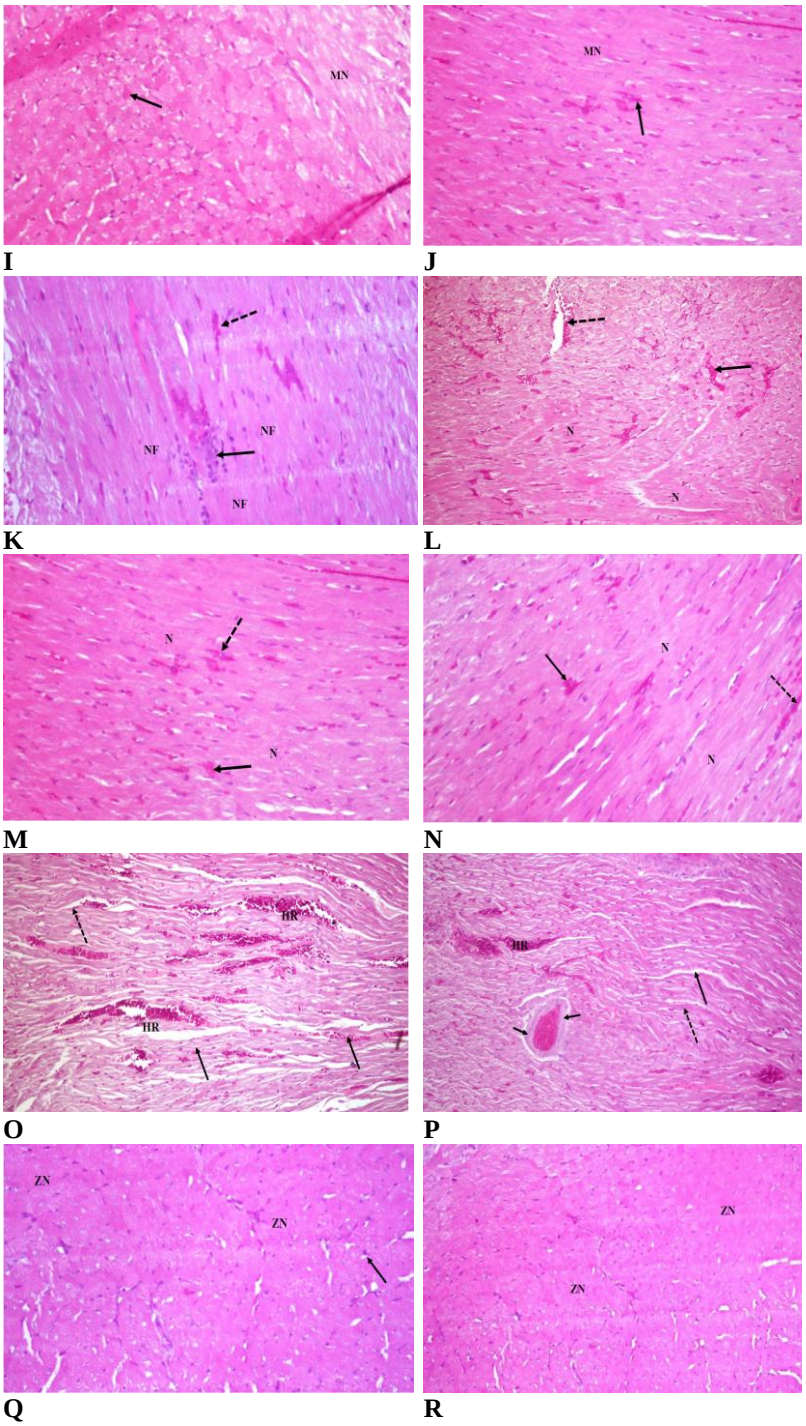


Fig. 7 The histological structure of the cardiac muscle infarcted heart fibers (A-R), all are 400×.

4. Conclusion

The goal of myocardial ischemia treatment is to improve blood flow to the heart muscle. Depending on the severity of your condition, your doctor may recommend medications, surgery or both. The study aimed to evaluate the possible cardio protective effect of new medications natural products from two different origin Vietnamese and Chinese ginseng (acidic, 80% methanolic and 70% ethanolic extract) against adrenaline-induced MI in rats. Oral pretreatment of all extracts showed a significant decrease in AST, LDH and CK-MB level as compared to control (MI) group. Histopathological examination of various treated groups (rats with infarcted hearts) showed moderated degree of myocardial necrosis and marked, scattered vascularization with rare intermuscular hemorrhages was a prominent finding.

Acknowledgment

Authors are grateful to the sincere support devoted by NRC.

Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

Abbreviation

List of abbreviation: MI: myocardial injury; DPPH: 1,1-diphenyl-2-picrylhydrazyl; ROS: reactive oxygen species; SEM: Scanning electron microscopy; EDAX: Energy Dispersive X-Ray Spectroscopy; TPC: Total phenolic content, GC/MS: Gas chromatography/mass spectrometry; TFC: Total flavonoid content; LSD: Least Significant Difference; EI: electron ionization; RI: retention indices; TCC: total carbohydrates content; PCA: Chemical composition-based main component analysis ; AHC agglomerative hierarchical clustering.

References

[1] Savic-radojevic, A., Pljesa-ercegovac, M., Matic, M., Simic, D., Radovanovic, S., Simic, T., 2017. Novel Biomarkers of Heart Failure. Adv Clin Chem, 79, 93-152 (2017).

[2] Robinson, C.F., Walker, J.T., Sweeney, M.H., Shen, R., Calvert, G.M., Schumacher, P.K., Ju, J., Nowlin, S. Overview of the National Occupational Mortality Surveillance (NOMS) system: leukemia and acute myocardial infarction risk by industry and occupation in 30 US states 1985-1999, 2003-2004, and 2007. Am J Ind Med. 58, 123-37(2015).

[3] Wybraniec, M., Mizia-stec, K., Krzych, L. Stress cardiomyopathy: yet another type of neurocardiogenic injury: 'stress cardiomyopathy'. Cardiovasc Pathol. 23, 113-20(2014).

[4] Aydin, S., Ugur, K., Aydin, S., Sahin, I., Yardim, M. Biomarkers in acute myocardiall infarction: current perspectives. Vasc. Health Risk Manag. 15, 1–10(2019).

[5] Thygesen, K., Alpert, J.S., Jaffe, A.S., Chaitman, B.R., Bax, J.J., Morrow, D.A., White, H.D., 2018. Executive group on behalf of the joint European society of Cardiology/American college of Cardiology/American heart association/world heart federation task force for the universal definition of myocardial I. Fourth universal definition of myocardial infarction. J. Am. Coll. Cardiol. 72 (18), 2231–2264(2018).

[6] Neri, M., Fineschi, V., Di Paolo, M., Pomara, C., Riezzo, I., Turillazzi, E., Cerretani, D., Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. Curr. Vasc. Pharmacol. 13 (1), 26–36(2015).

[7] Attalla, D.M., Ahmed, L.A., Zaki, H.F., Khattab, M.M., 2018. Paradoxical effects of atorvastatin in isoproterenol-induced cardiotoxicity in rats: role of oxidative stress and inflammation. Biomed. Pharmacother. 104, 542–549.

[8] Sethi, P., Peiris, C.D.,. A review of catecholamine associated cardiomyopathies and channelopathies. Cureus. 12 (2), e6957(2020).

[9] Hara, M.R., Kovacs, J.J., Whalen, E.J., Rajagopal, S., Strachan, R.T., Grant, W., Towers, A.J., Williams, B., Lam, C.M., Xiao, K., Shenoy, S.K., Gregory, S.G., Ahn, S., Duckett, D.R., Lefkowitz, R.J., A stress response pathway regulates DNA damage through beta2-adrenoreceptors and beta-arrestin-1. Nature 477 (7364), 349–353(2011).

[10] Diaz-Cruz, A., Guinzberg, R., Guerra, R., Vilchis, M., Carrasco, D., Garcia-Vazquez, F.J., Pina, E., Adrenaline stimulates H2O2 generation in liver via NADPH oxidase. Free Radic. Res. 41 (6), 663–672(2007).

[11] Mishra, S., Naaz, S., Ghosh, A.K., Paul, S., Ghosal, N., Dutta, M., Bandyopadhyay, D., Chattopadhyay, A.,. Orally administered aqueous bark extract of Terminalia arjuna protects against adrenaline-induced myocardial injury in rat heart through antioxidant mechanisms:an in vivo and in vitro study. J. Pharma Res. 10 (6), 454–478(2016).

[12] Andreadou, I., Iliodromitis, E.K., Farmakis, D., Kremastinos, D.T. To prevent, protect and save the ischemic heart: antioxidants revisited. Expert Opin. Ther. Targets 13 (8), 945–956 (2009).

[13] Radakovic, M., Borozan, S., Djelic, N., Ivanovic, S., Miladinovic, D.C., Ristani c, M., Spremo-Potparevic, B., Stanimirovic, Z.,. Nitroso-oxidative stress, acute phase response, and cytogenetic damage in Wistar rats treated with adrenaline. Oxidative Med. Cell. Longev. 21, 1805354(2018).

[14] Kharadi, G.B., Patel, K.J., Purohit, B. M., Baxi, S.N. Tripathi, C.B. Evaluation of cardioprotective effect of aqueous extract of Allium cepa Linn. bulb on isoprenaline-induced myocardial injury in Wistar albino rats. Res Pharm Sci, 11, 419-427(2016).

[15] Ligor, T., Gorczyca, P., Buszewski, B. Using Gas Chromatography for Indoor-Air Quality Control in Conservation and Renovation Studios, International Journal of Occupational Safety and Ergonomics, 11:3, 251-261, DOI: 10.1080/10803548.2005.11076647(2005).

[16] Wee, J.J., Park, K. M., Chung. An-Sik.,. Biological Activities of Ginseng and Its Application to Human Health. Herbal

Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis(2011).

[17] Zhang, H. M., Li, S. L., Zhang, H., Wang, Y., Zhao, Z. L., Chen, S. L., et al.. Holistic quality evaluation of commercial white and red ginseng using a UPLC-QTOF-MS/MSbased metabolomics approach. J. Pharm. Biomed. Anal. 62, 258–273(2012). doi:10.1016/j.jpba. 2012.01.010

[18] Chan, K. K. L., Yao, T. J., Jones, B., Zhao, J. F., Ma, F. K., Leung, C. Y., et al.. The use of Chinese herbal medicine to improve quality of life in women undergoing chemotherapy for ovarian cancer: A double-blind placebo-controlled randomized trial with immunological monitoring. Ann. Oncol. 22, 2241–2249(2011). doi:10.1093/annonc/mdq749

[19] Jiao, L., Dong, C., Liu, J., Chen, Z., Zhang, L., Xu, J., et al. Effects of Chinese medicine as adjunct medicatin for adjuvant chemotherapy treatments of non-small cell lung cancer patients. Sci. Rep. 7, 46524 (2017). doi:10.1038/srep46524.

[20] Ragab, I.M. T., Amer H., Wasfy A. A. F, Abdel Hady M. S., Abdel Tawab H., Liebner F.,. Sulfated Cellulose from Agriculture Wastes, Anticoagulant, Fibrinolytic and Toxicological studies. J. Environ. Sci. (7), 266-280(2014).

[21] Ragab I. M. T., Al Shimaa Gamal Shalaby, Sally A. El Awdan, Ahmed Refaat, Wafaa A. Helmy,. New applied pharmacological approach/trend on utilization of agro-industrial wastes Environmental Science and Pollution Research 25:26446–26460 (2018) https://doi.org/10.1007/s11356-018-2631-9

[22] Marrez, D.A., El Raey M. A., Ali M., Seif M. M., Ragab T.. I M., El Negoumy S. I. and Emam M. Phenolic profile and antimicrobial activity of green synthesized Acalypha wilkesiana seeds silver nanoparticles against some food borne pathogens. Biosci. Res. 14(4), 817–830(2017).

[23] Makkar, H.P.S.,. Measurement of Total Phenolics and Tannins Using Folin-Ciocalteu Method. In: Quantification of Tannins in Tree and Shrub Foliage. Springer, Dordrecht (2003). https://doi.org/10.1007/978-94-017-0273-7_3

[24] Saeed, N., Khan, M.R., Shabbir, M., Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complement Altern Med. 12, 221(2012).. https://doi.org/10.1186/1472-6882-12-221

[25] Ali, S.E., Farag, M.A., Holvoet, P., Hanafi, R.S., Gad, M.Z. A comparative metabolomics approach reveals early biomarkers for metabolic response to acute myocardial infarction, Sci. Rep. 6: 36359, https://doi.org/10.1038/ srep36359(2016).

[26] Broeckling, C.D., Reddy, I.R., Duran, A.L., Zhao, X., Sumner, L.W., MET-IDEA: data extraction tool for mass spectrometry-based metabolomics Anal Chem.78(13):4334-41(2006). doi: 10.1021/ac0521596.

[27] Reitman, S., Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol, 28, 56-63(1957).

[28] Abbot, B., Young, D.S., Burtis, A. Creatine kinase. In: Kaplan, A. (Ed.), Clinical Chemistry, edn. The C.V. Mosby Co., St Louis, Toronto, Princeton, pp. 1112–1116 (1984).

[29] Bancroft, J.D., Gamble, M.Theory and Practice of Histological Techniques. 6th Edition, Churchill Livingstone, Elsevier, China (2008).

[30] Mrigendra, M. Bastola, Craig Locatis, Richard Maisiak, Paul, Fontelo elenium, copper, zinc and hypertension: an analysis of the National Health and Nutrition Examination Survey (2011–2016). BMC Cardiovascular Disorders. 20, 45(2020).

[31] Shalaby, G. Al Shimaa, Ragab, I.M. T., Mehany B. A., Helal, M.I., Helmy, A. W., Antitumor and prebiotic activities of novel sulfated acidic polysaccharide from ginseng. Biocatalysis and Agricultural Biotechnology 14 402–409(2018).

[32] Malathy, R., Prabakaran, M., Kalaiselvi, K., Chung, I.-M., Kim, S.-H., Comparative Polyphenol Composition, Antioxidant and Anticorrosion Properties in Various Parts of Panax ginseng Extracted in Different Solvents. Appl. Sci. 11, 93(2021). https://dx.doi.org/10.3390/ app11010093

[33] Razgonova M.P. , Valery, V.V., Alexander, M. Z., Kirill, S. G., Alexander, E. N., G. C., Aristidis, T., Demetrios A. S., Panax ginseng components and the pathogenesis of Alzheimer's disease. Molecular Medicine Reports. 19(4): 2975–2998(2019). doi: 10.3892/mmr.2019.9972

[34] Ragab, I.M. T., Naglaa A. Ali, Abdel Nasser G. El Gendy, Safaa H. Mohamed, Aziza B. Shalby, Abdel-Razik H. Farrag, Al Shimaa Gamal Shalaby, Renoprotective and therapeutic effects of newly water, ethanol, and butanol ginseng fractions in hypertensive and chronic kidney disease with L-NAME.Biomedicine&Pharmacotherapy.142111 978(2021)https://doi.org/10.1016/j.biopha.2021 .111978.

[35] Wang, L., Zhao, X., Zhu, C., Xia, X., Qin, W., Li, M., Wang, T., Chen, S., Xu, Y., Hang, B., Sun, Y., Jiang, J., Richard, L.P., Lei, L., Zhang, G., Hu, J.,. Thymol kills bacteria, reduces biofilm formation, and protects mice against a

fatal infection of Actinobacillus pleuropneumoniae strain L20. Vet. Microbiol. 203, 202–210(2017).

[36] Salma A. El-Marasy, Sally A. El Awdan, Azza Hassan, Heba M.I. Abdallah,. Cardioprotective effect of thymol against adrenaline-induced myocardial injury in rats. Heliyon 6 e04431(2020).