The influence of grape seed extract on frog heart

**Abstract**

A grape seed extract has been used in many types of research in regard to showing its beneficial health effect due to its many active gradient contents. Often Some studies mention the benefits of using grape seed extract to improve heart function, in this investigation aqueous grape seed extract where used to see whether it’s acting on cardiac muscle by inotropic or chronotropic mechanism. **Material and methods**, aqueous grape seed extract where prepared after graining clean and dried grape seeds to obtain the powder, then the different watery solutions with different concentrations in percentages were prepared ( %5,%10,%15, %20,%25 ). After dissecting the frog heart from (the bufo genus ) and connecting it to power lab ML125 with a muscle transducer, using lab tutor software, the heart rate and mean changes percentage were obtained and calculated, by adding ( frog normal saline ) as baseline control and after ( using different concentration of grape seed extract ). **Results**, all of the prepared grape seed extract concentrations reveal increases in the mean heart rate and mean change percentage among the different concentrations %20 of grape seed extract reveal the highest impact on the mean heart rate ( 68.1 BPM ) compared to the frog normal saline ( 30.45 ), and the percentage of the mean change in the productivity heart rate was ( 123.3 % ) which may be related to calcium ion content which was ( 1.54 mg/dl ). In **conclusion** due to the high calcium ion content of the grape seed extract besides the bioactive gradient grape seed extract might has an inotropic effect on the cardiac muscle.

***Key words*** *. grape seed extract , calcium ion and inotropic effect , frog heart*

**Introduction**

Grapes (Vitis vinifera) are one of the oldest known beneficial nutritional components of the human diet (Troilo et al., 2021). Grape seeds are rich in proanthocyanidins which have been shown to possess potent free radical scavenging activity. Grape seeds are a complex matrix containing 40% fiber, 16% oil, 11% proteins, and 7% complex phenols such as tannins (Ma and Zhang, 2017). Grape seeds contain fats, proteins, carbohydrates, and 5–8% polyphenols. The grape seed is rich in extractable phenolic antioxidants such as phenolic acids, flavonoids, proanthocyanidins, and resveratrol, and the grape skin is abundant in anthocyanins (Gómez-Mejía et al., 2021). Grape seed extract does possess a cardioprotective effect, for example, it reduces the likelihood of heart attack (Chang et al., 2021) . Furthermore, it is also beneficial for numerous other cardiovascular diseases. Quercetin inhibits the oxidation of LDL and cholesterol and the clumping of platelets. Other beneficial effects are exerted by resveratrol, epicatechin, epigallocatechin gallate (EGCG), epicatechin gallate (ECG), genistein, and daidzein, namely, they protect against atherosclerosis and alleviate arrhythmias of the heart (Li et al., 2022). Polyphenols reduce the risk of cardiovascular disease, for example, they decrease the risk of coronary heart disease. They act by dilating the blood vessel walls, thereby reducing blood pressure (Kolahdouz-Mohammadi et al., 2021, Sun and Li, 2022) (Sun and Li, 2022).Evidence from in vitro experiments suggests that grape seed extract has an antioxidant property that can protect cells from ROS-mediated DNA damage (Li et al., 2010). grape seed extract is commercially available on the market, and it is generally well tolerated when taken by mouth. Several clinical trials have reported a beneficial impact of grape seed extract on blood pressure (Sivaprakasapillai et al., 2009) (Ras et al., 2013). Meanwhile, other researchers have not found any relation or impact of grape seed extract on blood pressure (Dohadwala et al., 2010) (Siasos et al., 2014). Grape seed extract has been shown in clinical trials to increase satiety, reduce energy intake from food intake, and increase fat breakdown in vitro (Vogels et al., 2004). Grape seed extract inhibits the enzymes involved in fat metabolism (pancreatic lipase, lipoprotein lipase), thus preventing the accumulation of fat in adipose tissue (Moreno et al., 2003). Mice fed grape seed extract have reduced tissue fat levels but not influenced body weight (Mittal et al., 2003). Polyphenols isolated from grape seeds and red wine inhibit intracellular cholesterol synthesis, and thereby reduce blood cholesterol level (Leifert and Abeywardena, 2008b). In other hand Oral administration of a grape seed extract reduces the oxidative stress, inflammation and atherosclerosis in a rat model of ligature-induced periodontitis (Dimitriu et al., 2020).

**Materials and methods**

Plant Materials Seeds from the grapes were collected from local markets in Erbil. seeds were washed with distilled water and dried in shade for at least 10 days, and then all parts of the grape seeds were grinded to a fine powder and stored in an airtight container at room temperature in the dark until used (Sharma et al., 2014).

The powdered samples were subjected to extraction using the water as a solvent following the method of Nair et al. dissolving 5 g , 10 g , 15g , 20 g, and 25 g of grape seed powder samples in 100 ml distilled water in sterile wide-mouthed screw-capped bottles of 250 ml volume then allowed to soak for 24 hrs at 4C (Nair et al., 2005).

**Frog animal dissection**

Obtain a double-pithed frog (the bufo genus ) then Place the frog on its dorsal surface. Using the sharp end of a pair of blunt/sharp scissors make a small penetration into the abdominal cavity of the frog. Carefully cutting the abdominal wall with a pair of scissors and cut towards the sternum. Lift the scissor as we cut to prevent cutting off the heart blood vessels or other internal organs. Cutting through the pectoral girdle to expose the heart in the pericardial sac. Carefully remove the pericardial sac. Cutting any connective tissue attachments (not the vessels around the atria) so that the heart beats freely Using forceps to grasp the apex of the ventricle and push the point of the ‘J’-hook into the apex of the ventricle. Push the hook through the ventricle wall until the bend of the hook is inside the heart. Connect the thread from the frog heart to the force transducer S-hook Use a square knot and tie it twice to make sure it won’t come to loosen when the heart contracts. Position the frog so the thread from the heart is vertical. If it is pulling at an angle much of the contraction of the frog heart will not be observed.

## PowerLab Setup ML 125

1. Connect the force transducer 5 to 50 gm cable to the back of the Bridge Pod.
2. Attach the Bio Amp cable to the Bio Amp socket on the PowerLab.

## Other Equipment & Materials

* Dissecting dish.
* Straight metal pins.
* Dissecting equipment: Scissors, forceps, etc.
* Strong thread with a sharp barbless hook.
* Room temperature, warm and cold Frog Ringer's solution's.

Frog or Amphibian Ringer’s solution (6.6 g NaCl, 0.15 g KCl, 0.15 g CaCl2, 0.2 g NaHCO3 in 1 L of distilled water) is effective; alternatively, a solution of equal parts 2.5% dextrose in 0.45% sodium chloride and lactated Ringer’s may be more readily available and can be used.

* Acetylcholine solution (0.1 mg/mL) .
* Stimulator rod.

Applying the GSE in this experiment in the order indicated. Between each step, allow the heart to recover for two minutes and flush with fresh Frog Ringer's solution.

**Grape seed extracts with different concentration effects on frog heart**

1. Click Start and record 30 seconds of baseline data.
2. Using a syringe, apply two or three drops of 5% GSE to the heart. Add a Comment GSE .
3. Record for one minutes.
4. Click Stop.
5. Rinse the heart with Frog Ringer's solution and allow the heart 2 minutes to recover.
6. Repeat step 2 for each concentration 10%, 15% ,20% and 25 % of GSE

**Grape seed extract with different concentration and Acetylcholine**

1. Click Start and record 30 seconds of baseline data.
2. Using a syringe, apply two or three drops of GSE (20 % ) to the heart. Add a comment GSE.
3. Record for 30 seconds.
4. Apply two or three drops of acetylcholine to the heart. Add a Comment 'ACh'. (0.1 mg/mL)
5. Record for two minutes.
6. Click Stop.
7. Rinse the heart with Frog Ringer's solution and allow the heart 2 minutes to recover.

## Analysis the obtained data

1. For each drug administered use the Waveform Cursor to determine the heart rate just before exposure and at the end of exposure to the drug, or drugs.
2. Click to enter each heart rate into the Value panel and then drag the rate to the appropriate cell in the table.
3. The table will show the percentage change in heart rate calculated using the equation:

**The results**

The obtained Results in the tables ( 1,2, and 3 ) with figures ( 1 and 2 ) shows all of the prepared grape seed extract with different concentration reveals increases in the mean heart rate , and mean change percentage among the different concentration %20 of grape seed extract grape seed extract has the highest impact on the mean hear rate( 68.1 BPM ) compared to the frog normal saline ( 30.45 ) , and the mean changes percentage in the productivity of the heart rate was ( 123.3 % ) which might be related to calcium ion content which was ( 1.54 mg/dl ).

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| **table 1: shows the effect of different concentration percentages of grape seed extract ( G.S.E )on the mean heart rate and the mean changes percentage between before and after.** |
| **concentration** | **mean heart rate** | **mean changes % between before and after** |
| frog normal saline | 30.45 | \_\_\_\_\_ %0  |
| %5 G.S.E | 46.05 | 51% |
| 10% G.S.E | 60.3 | 98.00% |
| 15% G.S.E | 60.4 | 98.30% |
| 20% G.S.E | 68.1 | 123.30% |
| 25% G.S.E | 61.9 | 103.30% |

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| **table 2: shows the effect of acetylecholine and grape seed extaract on the mean heart rate and the changes percentage .** |
| **concentration**  | **mean heart rate**  | **mean change percentage before and after**  |
| frog normal saline  | 30.4 | \_ |
| acetyle cholin 1% | 24.1 | -20.70% |
| 5% | 83.4 | 174.30% |
| 10% | 67.6 | 122.40% |
| 15% | 46.4 | 52.60% |
| 20% | 81.2 | 167.10% |
| 25% | 63.6 | 109.20% |

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| **table 3: shows the concentration of total calsium ion in mg/dl for different concentrations of grape seed extract.** |
| **number**  | **different concentration of grape seed extract**  | **total calsium content in mg/dl** |
| 1 | 5% | 0.73 |
| 2 | 10% | 0.93 |
| 3 | 15% | 1.15 |
| 4 | 20% | 1.54 |
| 5 | 25% | 2.26 |

**Discussion**

Grape (Vitis vinifera L.) is one of the most popular fruits worldwide. It contains various bioactive compounds, such as proanthocyanidins, anthocyanins, flavonols, phenolic acids and stilbenes, the contents of which could vary considerably in grape skin, pulp and seed. Many studies have revealed that grape possesses a variety of health benefits, such as antioxidant, anti-inflammatory, gut-microbiota-modulating, anticancer and cardioprotective effects. Grape is eaten as fresh fruit and is also used as raw material to produce various products, such as grape juice and raisins. Moreover, the byproducts of grape, such as grape pomace and grape seed, have many applications in the food industry. In this paper, the bioactive compounds in grape are briefly summarized based on literature published in recent years. In addition, the health benefits of grape and its bioactive components are discussed, with special attention paid to the underlying mechanisms (Zhou et al., 2022). A regular intake of red grape juice has cardioprotective properties, but its role on the modulation of natriuretic peptides (NPs), in particular of C-type NP (CNP), has been proven (Svezia et al., 2020). Cardiovascular disease is the leading cause of death worldwide (Nunes et al., 2016). Grape and its bioactive compounds have been reported to exert excellent efficacy in the prevention and treatment of cardiovascular disease. Hypertension is among the most prominent risk factors for cardiovascular disease (Ebrahimi-Mameghani et al., 2020). Grape exhibits hypotensive activity, which could effectively prevent the development of cardiovascular diseases. For example, in vitro and in vivo experimental results showed that grape extract from chardonnay (a grape cultivar for white wine) increased nitric oxide (NO) production in cultured endothelial cells and ameliorated endothelial dysfunction and hypertension induced by deoxycorticosterone acetate-salt in rats by activating the endothelial NO synthase and PI3K/Akt pathways (Sato et al., 2020). In addition, grape seed proanthocyanidin ameliorated left ventricular remodeling in spontaneously hypertensive rats by lowering SBP, reducing oxidative stress and regulating the levels of vasoactive substances (Liu et al., 2021). Myocardial infarction is a severe and common type of cardiovascular disease caused by acute occlusion of coronary arteries. The results of a study by Svezia et al. suggest that pure grape juice protected the myocardium from an ischemic microenvironment and protected infarcted hearts by regulating gene expression of C-type natriuretic peptide, which is among the major autocrine/paracrine regulators of cardiac remodeling in infarcted hearts, acting as an endothelium-derived hyperpolarizing factor, modulating vascular tension and blood flow (Svezia et al., 2020). In addition, grape seed proanthocyanidin extract effectively improved cardiac remodeling and dysfunction caused by myocardial infarction in mice and protected cardiomyocytes from apoptosis in hypoxic situations through the PI3K/Akt pathway (Ruan et al., 2020).

Dried seeds of grapes contain around 35% fiber along with 29% extractable components including Phenolic compounds, proteins (11%), mineral (3%) and water (7%) (Matthäus, 2008).

Some research found that GSE may be beneficial for individuals with or at risk of cardiovascular disease because it may have hypotensive and HR-lowering properties (Foshati et al., 2022). flow-mediated dilation (FMD) was significantly improved following supplementation with GSE in some trials (Barona et al., 2012). but was not significantly affected in others (Kim et al., 2012). However, in Ward et al.'s study, 6-week supplementation with 1000 mg/day GSE and 500 mg/day vitamin C significantly increased SBP and DBP compared with the control group, who received placebo and vitamin C (Grundy et al., 2001). In spite of these findings, no significant beneficial or detrimental effects were observed on SBP and DBP in some other trials (Leifert and Abeywardena, 2008a), (Faisal et al., 2015) . these finding supporting or results about the inotropic effect of grape seed extract on increasing the heart rate which may be due to the high content of the calcium ion in that we obtained in the grape seed extract . grape seeds were thought to be important sources of nutrients and essential elements , As well as, grape seeds also contain many minerals, including calcium (0.56%) (Hajati et al., 2015). This is might be the main factor which leads to increasing in the heart rate of the frog animal after adding different concentration of warty extract of GSE as its shown obviously in the results of this study , Calcium sensitizers increase myocardial contractile function without affecting Ca2+ homeostasis, which might be beneficial in the treatment of patients with heart failure (Tsutsui et al., 2001).

To confirm the inotropic effects of grape seed extract acetylcholine used as an antagonist agent , as its obtained in the study results The mean changes in the heart rate and the percentage change in the heart rate were decreased significantly after the application of acetylcholine to the atria of an isolated heart of a frog produces a specific negative chronotropic effect without decreasing its amplitude (Kudrin and Zhdanova, 1976). Then after adding the grape seed extracts with effective concentration the heart rate increased with the percentage in the mean changes . Calcium (Ca2+) is a critical regulator of cardiac myocyte function. Principally, Ca2+ is the link between the electrical signals that pervade the heart and contraction of the myocytes to propel blood. In addition, Ca2+ controls numerous other myocyte activities, including gene transcription. Cardiac Ca2+ signaling essentially relies on a few critical molecular players—ryanodine receptors, voltage-operated Ca2+ channels, and Ca2+ pumps/transporters. These moieties are responsible for generating Ca2+ signals upon cellular depolarization, recovery of Ca2+ signals following cellular contraction, and setting basal conditions. Whereas these are the central players underlying cardiac Ca2+ fluxes, networks of signaling mechanisms and accessory proteins impart complex regulation on cardiac Ca2+ signals (Fearnley et al., 2011).

**Conclusion**

Grape is one of the most produced and consumed fruits in the world, representing a source of polyphenols, especially proanthocyanidins, anthocyanins , resveratrol and many minerals . The calcium ion rich contents considerable as important factor in the extract which paly a role in the enhancement and regulation of the heart function which may be due to the inotropic effects on the heart . In the future, more bioactive compounds in grape should be isolated and identified. Their bioactivities should be evaluated, and their underlying mechanisms of action should be explored.

Acknowledgments

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