



The impact of almond seeds aqueous extract on frog heart

Research project

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Abstract

Because almond seeds include so many active gradient components, they have been employed in a variety of studies to demonstrate their positive health impacts. Aqueous almond seed extract was used in this study to determine whether the benefits of using almond seeds as a natural dietary supplement to improve heart function by lowering systolic blood pressure and total cholesterol were due to inotropic or chronotropic mechanisms. Materials and procedures: First, clean, dried almond seeds were ground into a powder that resembled a dry paste. Next, various watery solutions with varying percentage concentrations were made (10%, 5%, 2.5%, and 1.25%). Finally, aqueous almond seed extract was created. Lab tutor software was used to calculate the heart rate and mean changes percentage after the frog heart from the Bufo genus was dissected and connected to a Power Lab ML125 using a muscle transducer. Frog normal saline was added as a baseline control, and different concentrations of almond seed extract were added thereafter. Findings indicate that the mean heart rate and mean change percentage (%10) of the various manufactured almond seed extract concentrations (A.S.E) are all lower than before. indicate that the greatest effect was seen in the mean heart rate, which dropped to 42 BPM from the frog's usual salinity of 66, and the percentage of the mean change in the cardiac productivity rate dropped to (-36.3%), which may be connected to potassium ion. In conclusion, if almond seed extract is ingested in large quantities throughout the day, it may have a chronotropic effect on the heart muscle in addition to its bioactive gradient because of its high potassium ion content.

Key words. almond seed extract, potassium ion, and chronotropic effect, frog heart

Introduction

Due to its nutritional and medicinal qualities, the sweet almond (*Prunus dulcis* Miller D.A. Webb) is a well-known nut that has been utilized for a long time in a variety of ethnomedical systems, most notably Persian medicine (PM).

nutritional benefits of almonds, uses for almond kernel and oil, and total phenolic, flavonoid, and antioxidant activity as well as fatty acid profile and polyphenols. About 24–73% crude oil, 50–84% oleic and 6–37% linoleic acids, 77–3908 mg/kg β -stosterol, and 5–8 mg/100 g β -tocopherol were found in almonds' composition. Almonds have therapeutic properties and are a good source of vitamins E, minerals, phytochemicals, unsaturated and monounsaturated fatty acids, polyphenols, and phytosterols (Özcan, 2023).

Phytochemical studies reveal that this plant has a variety of phenolic compounds, essential oils, macro- and micronutrients, and phytosterols. *Prunus dulcis* is reported to possess a variety of biological activities, including nootropic, anxiolytic, sedative-hypnotic, hepatoprotective, anticancer, antioxidant, antibacterial, and anti-inflammatory properties. These findings are supported by current pharmacological investigations.

and benefits on the neurological system (Karimi et al., 2021). Almonds include a variety of nutrient-dense components, including fatty acids, lipids, amino acids, proteins, carbohydrates, vitamins, and minerals, as well as secondary metabolites, according to studies on the composition and characterisation of almond macro- and micronutrients (Barreca et al., 2020).

Only walnuts, however, have demonstrated encouraging outcomes in empirical trials for memory enhancements, followed by almonds, hazelnuts, and pistachios. The purpose of this review is to give theories about the anti-dementia properties of nine distinct types of nuts: chestnut, cashew, hazelnut, almond, walnut, pistachio, peanut, Brazil nut, and pecans. Essential fats (primarily mono- and polyunsaturated fatty acids), proteins (sources of arginine, lysine, and tryptophan), vitamins (riboflavin, folate, and various tocopherols), fibers, minerals (calcium, sodium, magnesium, phosphorus, and potassium), and trace elements (copper, zinc, and selenium) are all included in nuts' nutritional profile. It's interesting to note that natural product ingredients—nuts being a prime example—function in concert or to offset adverse effects (Arslan et al., 2020).

Almonds have a high content of unsaturated fatty acids, little saturated fat, fiber, phytosterols, and plant protein. Almonds are a unique source of various cardioprotective elements, such as α -tocopherol, arginine, magnesium, copper, manganese, calcium, and potassium. The nutrients that almonds offer are probably linked to the mechanisms that cause the LDL-C lowering that is seen with almond eating (Berryman et al., 2011).

While keeping HDL-C concentrations constant, almonds decreased central adiposity, LDL-C, and non-HDL-C, three significant risk factors for cardiometabolic dysfunction. Consequently, eating 1.5 ounces of almonds per day in place of a high-carb snack may be a straightforward dietary approach to delay the onset of cardiometabolic illnesses in otherwise healthy people (Berryman et al., 2015).

Potassium and phosphorus are the two most prevalent mineral elements found in almonds; when nitrogen is excluded, these macronutrients account for more than 70% of the total mineral component. Sodium, iron, copper, manganese, and zinc are examples of microminerals. Tocopherols, squalene, phytosterols, stanols, phospholipids, sphingolipids, chlorophylls, carotenoids, phenols, and volatile chemicals are among the phytochemical substances (Roncero et al., 2020).

Twenty patients with mild hypercholesterolemia (mean age 35 years, mean BMI 23, and 12 men and 12 women) were randomized in a crossover RCT to receive their usual diet plus 20 g (109 kcal) of baru almonds or a corn starch placebo capsule for a period of six weeks. When compared to the placebo, the baru almond-enriched diet tended to lower body mass, BMI, and body fat percentage (Bento et al., 2014).

The presence of phenolic compounds, specifically vanillic, caffeic, p-coumaric, and ferulic acids (after basic hydrolysis), quercetin, kaempferol, and isorhamnetin (after acidic hydrolysis), delphinidin and cyanidin (after n-butanol-HCl hydrolysis), and procyanidin B2 and B3 was revealed by high-performance liquid chromatography (HPLC) analysis of almond seed crude extract (Amarowicz et al., 2005).

Materials and methods

Plant Substances The almond seeds were gathered from Erbil's neighborhood markets. All of the almond seeds were ground into a fine powder and kept in an airtight container at room temperature in the dark until needed. The seeds were then cleaned with distilled water and dried in the shade for at least ten days (Sharma et al., 2014). After dissolving 10 g, 5 g, 2.5 g, and 1.25 g of almond seed powder samples in 100 ml of hot distilled water in sterile wide-mouthed screw-capped bottles of 250 ml volume, the powdered samples were extracted using the hot distilled water at (70 Co) as a solvent. The samples were then allowed to soak for 24 hours at 4C (Nair et al., 2005).

Frog animal dissection

In this experiment, a group of seven male frogs with a narrow weight range was used. The frog (*Bufo bufo*), often known as the common or European toad, was then placed on its dorsal surface using a double-pithing procedure. Make a little incision into the frog's abdominal cavity with the sharp end of a pair of blunt or sharp scissors. Using a pair of scissors, carefully cut the abdominal wall in the direction of the sternum. As we cut, raise the scissor to avoid severing the blood arteries in the heart or other internal organs. cutting open the pericardial sac to reveal the heart beneath the pectoral girdle. Remove the pericardial sac with caution. removing any connective tissue attachments to allow the heart to beat freely (not the veins surrounding the atria). inserting the point of the "J"-hook into the ventricle's apex after grabbing it with forceps. Once the hook's bent is within the heart, push it through the ventricular wall. Attach the force transducer S-hook to the frog heart's thread. To ensure that it won't come free when the heart contracts, tie it twice using a square knot. Arrange the frog such that the heart's thread is vertical. A large portion of the frog heart's contraction won't be visible if it is tugging at an angle.

Power Lab Setup ML 125

1. Attach the 5 to 50 gm force transducer cable to the Bridge Pod's rear.
2. Connect the Bio Amp cable to the Power Lab's Bio Amp socket.

Other Equipment & Materials

1-Examining a dish.

2-Linear metal fasteners.

3-Dissecting tools: forceps, scissors, etc.

4-Robust thread featuring a sharp, barbless hook.

5-The temperature of the room, both warm and cold Frog Ringer solutions.

Ringer's solution for frogs or amphibians (6.6 g NaCl, 0.15 g KCl, 0.15 g CaCl₂, 0.2 g NaHCO₃) in 1 L of distilled water works well. Alternatively, lactated Ringer's with equal parts 2.5% dextrose in 0.45% sodium chloride may be easier to find and use.

6-The stimulator rod. Using the almond seed extract (ASE) in this experiment according to the suggested sequence. Give the heart two minutes to rest between steps and then flush with new Almond seed extracts with different concentration effects on frog heart Frog Ringer's solution.

Almond seed extracts potassium estimation

The measure Spectroquant® Potassium Photometric Test 5.0 – 300 mg/l K – USA was used to the potassium contents in (mg/dl) using the various watery extracts of the different concentrations.

Approach: photometric

The potassium concentration of aqueous samples can be accurately measured with the help of the Spectroquant® Potassium Cell Test.

Technique used: Potassium ions and Kalignost® (sodium tetraphenylborate) combine in an alkaline solution to generate a somewhat soluble precipitate. The photometer is used to measure the turbidity that results (turbidimetric method).

Almond seed extracts with different concentration effects on frog heart

1. Press Start to capture baseline data for 30 seconds.
2. Apply two or three drops of 10% ASE to the heart using a syringe. Post a Comment, GSE.
3. Keep a one-minute record.
4. Press the Stop button.
5. After giving the heart a 2-minute rest, rinse it with Frog Ringer's solution.
6. For each concentration of ASE—10% ,5%, 2.5%, and 1.25 %—repeat step 2.

Analysis the obtained data

1-Use the Waveform Cursor to measure heart rate both before and after exposure to any given drug or combination of substances.

2-Click in the Value panel to enter each heart rate, and then drag the rate to the relevant cell in the table.

3.The table will appear the rate alter in heart rate calculated utilizing the condition:

Percent alter in heart rate = (Contrast between the two heart rates)/(Original heart rate) x 100.

The results

According to the results obtained, the prepared almond seed extract with different concentrations shows a decrease in the mean heart rate. The mean change percentage among the different concentrations of almond seed extract shows that 10% A.S.E. has the highest impact on the mean heart rate (BPM) compared to the frog normal saline concentration of 30.45. The mean changes percentage in the productivity of the heart rate decreased negatively to (-36.3%), which may be related to the potassium ion content, which was shown in table (2) and figure (3).

table 1: shows the effect of different concentration percentages of almond seed extract (A.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	66	_____ %0
10% A.S.E	42	-36.3
5% A.S.E	48	- 27.2
2.5% A.S.E	54	-18.1
1.25% A.S.E	60	-9.09

table 2: shows the concentration of the total potassium ion in mg/dl for different concentrations of almond seed extract.		
number	different concentration of almond seed extract	total potassium content in mg/dl
1	10%	74.2
2	5%	34.3
3	2.5%	18.1
4	1.25%	8.54

Discussion

It is repeatedly demonstrated by randomized controlled experiments that nuts decrease cholesterol. Consuming nuts also somewhat reduces inflammation, blood pressure (BP), endothelial function, and glycemic management. Despite being high in energy, nuts do not increase the risk of obesity and may even aid in weight loss. Peanuts and tree nuts, but not peanut butter, typically have comparable beneficial benefits on results. According to preliminary data from the PREDIMED trial, eating 30 g/d of nuts (walnuts, almonds, and hazelnuts) within the framework of a Mediterranean diet significantly reduced the risk of a composite endpoint of major adverse cardiovascular events (heart attack, stroke, and cardiovascular death) by about 30% over the course of a 5-year intervention. Remarkably, a diet rich in nuts lowered the risk of stroke by 45 percent. Nuts can be regarded as natural pleiotropic nutraceuticals because of their abundance in salutary bioactive components and positive effects on a range of health outcomes (Ros et al., 2021).

Increased potassium intake lowers blood pressure in hypertensive individuals and has no negative effects on adult blood lipid, catecholamine, or renal function levels, according to high-quality research. A 24% decreased risk of stroke was linked to higher potassium intake (moderate grade evidence). According to Abboto et al. (2013), these findings imply that most individuals without impaired renal processing of potassium may benefit from increased potassium consumption in terms of preventing and controlling high blood pressure and stroke.

Per 100 grams of weight, almonds contain 705 milligrams of potassium (Richardson et al., 2009). Many of the early electrophysiological alterations are caused by an increase in the concentration

of potassium ions outside of cells. Arrhythmias can be caused by electrical forces that are created by sudden variations in the plasma potassium concentration in the normal myocardium and by high potassium concentration in the ischemic myocardium (Poole-Wilson, 1984).

Recent RCTs point to potential new health benefits of almonds, including improved heart rate variability and improved cognitive function under mental stress (Dreher, 2021). In diabetic ketoacidosis, mild to severe elevations in serum potassium are common. Severe hyperkalemia, on the other hand, is rare and most commonly caused by acidosis, hyperosmolality, severe dehydration, insulin insufficiency, and renal potassium retention. Manappallil and Nambiar (2020) state that immediate correction is necessary to avert cardiac arrest due to an excessive level of potassium.

A diet that increases potassium intake while modestly restricting salt intake is a strategy to prevent or control hypertension and lower the morbidity and death rate associated with cardiovascular disease. Therefore, as an important public health initiative to avoid kidney

disease, stroke, and cardiovascular disease, the body of evidence supports population-wide reductions in sodium intake and suggested increases in dietary potassium intake as described by current guidelines (Aaron and Sanders, 2013).

The correct assessment of many clinical potassium abnormalities requires an understanding of the medicines that impact potassium homeostasis, either by changing the renal excretion of potassium or by changing its distribution. Both hyperkalemia and hypokalemia have the potential to result in fatalities, severe arrhythmias, asymptomatic ECG abnormalities, and muscle weakness (Brown, 1984).

The serum potassium level was linked to hemodynamics and heart function in the early postresuscitation period. A higher potassium level was linked more frequently to acidosis, bradycardia, nonsinus rhythm, and urine production less than 1 ml/kg/hr.

More crucially, according to Lin et al. (2018), a high potassium level shortened the survival period. Lin and others. It was discovered that individuals with high potassium levels (41.4%) experienced bradycardia more frequently than those with low (16.7%) or normal (18.0%) potassium levels.

A high potassium level was strongly associated with slower mean heart rates as compared to low or normal levels, particularly during the first half hour of the postresuscitation period. The heart rate variations between low, normal, and high levels were not statistically significant after 30 minutes (time points 31–45 and 46–60 minutes).

This result suggested that potassium levels might undoubtedly affect the dynamic variation in heart rate, particularly during the first half hour. Therefore, during this time, it is important to focus on constant observation and active treatment to prevent cardiac arrhythmias related to hyperkalemia.

An increased risk of cardiovascular death was linked to elevated serum potassium levels (CVM). Elevated blood potassium was associated with an increased risk of ventricular arrhythmias in patients suffering from acute myocardial infarction. In patients with chronic kidney disease and dialysis, hyperkalemia was the only factor linked to an elevated risk of a composite cardiovascular outcome (Hoppe et al., 2018).

Conclusion

One of the most widely grown and consumed fruits in the world, almonds are rich in proanthocyanidins, anthocyanins, resveratrol, and other minerals, as well as polyphenols. The extract's high concentration of calcium ions is a significant component that contributes to the improvement and control of cardiac function. This may be because the extract has inotropic effects on the heart. More bioactive components from almonds should be found and identified in the future. It is important to assess their bioactivities and investigate the underlying mechanisms of action.

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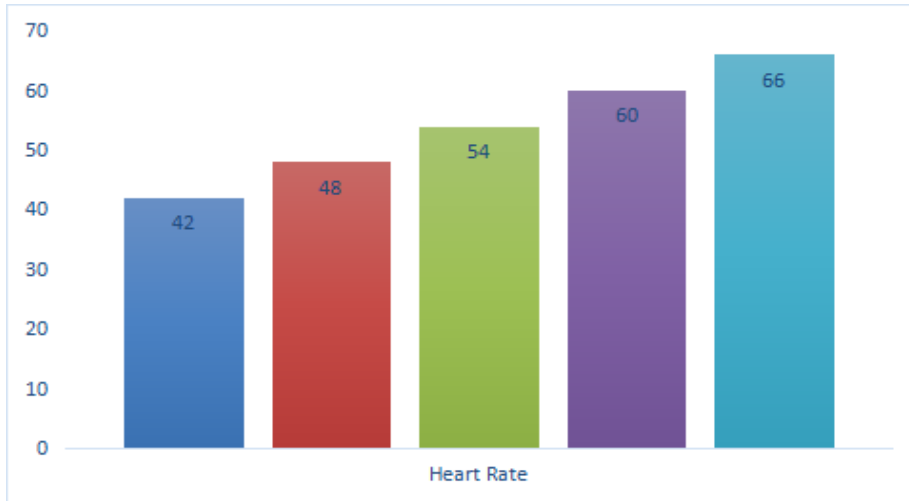


figure 1: shows the changes of the mean heart rate after using different percentage concentration of almond seed extract (A.S.E)compared to the baseline of the frog saline.

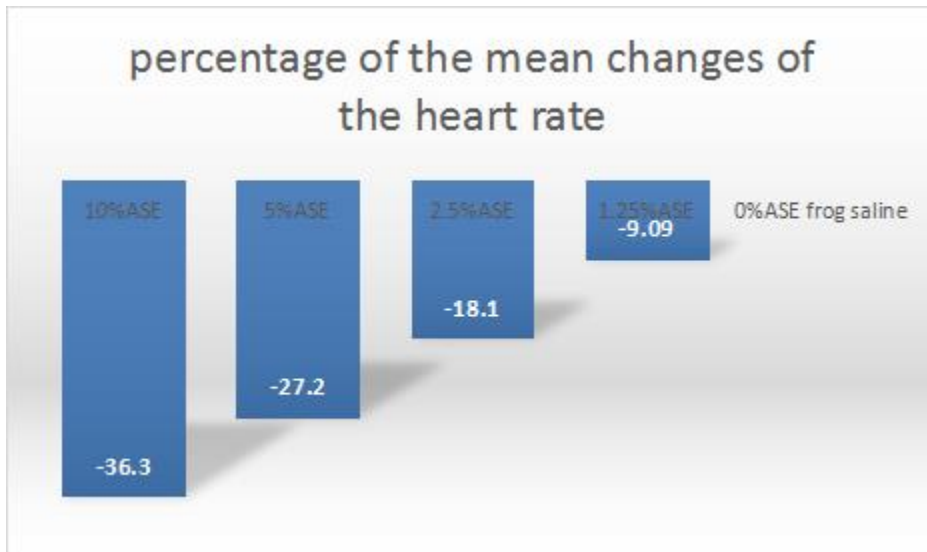


figure 2: shows the percentage of the mean changes of the heart rate after using different concentration of grape seed extract compared to the bassline of the frog normal saline.

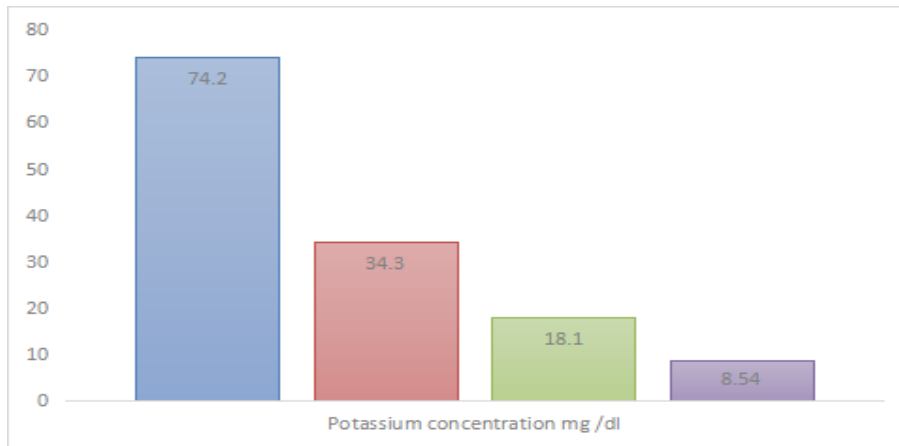


figure 3: shows the potassium concentrations (mg/dl)in different percentage of prepared almond watery extracts.

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