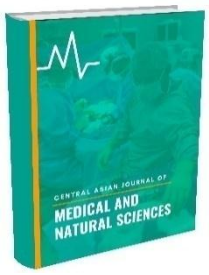
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 06 | Nov-Dec 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Treatment of Acute Myocardial Infarction in Rats Using Flavonoids Extracted From Bay Leaf

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Received 2nd Oct 2023,
Accepted 19th Nov 2023,
Online 21st Dec 2023

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Abstract: This research included evaluating the levels of prolidase enzyme and sodium in a group of rats exposed to myocardial infarction caused by subperitoneal injection of physiological solutions after fasting for 18 hours. The mice were divided into five groups. The first group was a healthy control group, the fifth group received the drug simvastatin, and the remaining groups were given different concentrations of flavonoids (extracted from the bay leaf plant using HPLC technology).

Key words: Bay leaf plant, Myocardial infarction, Prolidase Enzyme, Sodium.

Bay leaf plant Evergreen trees, which have economic and commercial benefits from their essential oils, which are extracted from the leaves. They have small, black, oval fruits with a thick, peeling outer covering, containing a single seed [1]. The main phenolic compounds found in laurel extracts are flavonoids, especially luteolin, and phenolic acids such as Vanillic acid and Rosmarinic acid [2].Gallic acid. Laurel appeared as a remedy to relieve the symptoms of influenza and bronchitis and to help with digestive system problems, insomnia, and menstrual pain [3]. Bay leaves are used to relieve pain, especially stomach and intestinal pain. It is also considered a local anesthetic and is effective against rheumatic diseases and skin rashes [4]. **Myocardial ischemia** When the coronary arteries are blocked, one of the forms of acute coronary syndrome occurs[5]. It can be brought on by either a decrease in oxygen supply or an increase in oxygen demand, as evidenced by coronary artery spasm and occlusion [6], as well as unexpected sudden cardiac death, such as cardiac arrest. It frequently manifests with symptoms that point to myocardial ischemia, like coronary artery blockage [7]. a fresh coronary blood clot following a previous injury, which has the potential to produce ventricular fibrillation, the primary cause of sudden cardiac death[8]. **Prolidase Enzyme** is a polypeptide with a metal base that is expert at cleaving dipeptides. It has the capacity to break the bonds present in the Glycyl-L-proline complex and is composed of (493) amino acids[9].It is crucial for cell development, matrix remodeling, and collagen metabolism. Prolidase's ideal substrate is glycine-L-proline. Manganese serves as the enzyme's catalyst [10, 11]. **Sodium** is one of the main ions of fluids outside the body's cells and is necessary for the functions of the nerves, muscles, and stomach as a whole. It is the necessary ion that maintains the water balance and the appropriate pH of the blood[12],and the normal level of it in the blood serum is (145-135) grams/liter [13] .In addition, sodium maintains the acid-base balance in the body and maintains the electrical potential of cell membranes [14] ,and the exchange that occurs

between the sodium ion and the potassium ion helps in cell function as it maintains water balance and neuromuscular activities [15].

MATERIALS AND METHODS

The experimental animals were divided and distributed homogeneously in terms of weight into 5 groups, 5 animals for each group. The animals were treated once daily for 21 days with the treatments specific to the experiment, as follows: The first group was treated with Triton x-100, the standard diet, and distilled water, and it was considered an infected control group. As for the fifth group, it was treated with the standard diet and distilled water and was considered a healthy control group, while the remaining groups of animals were treated with special treatments by dosing them orally.

Serum preparation

After the end of the specified period of 21 days for the experiment, the animals were anesthetized using chloroform to collect blood samples from the eye hole, and they were placed in blood collection tubes containing an anticoagulant gel tube to conduct an examination ((CBC Complete blood count), while the remaining part was placed in test tubes. tubes) free of anticoagulant, and then the serum was obtained by centrifuging it using a centrifuge at a speed of 6000 rpm for 15 minutes, to obtain the serum that was stored in a 2ml Eppendorf tube at a temperature of (10-20°C) until the tests are conducted.

Extraction of flavonoids

Bay leaf and rosemary were extracted using 70% ethanol, and upon evaporating the ethanol, a crude product was obtained that was subjected to diagnosis by high-performance chromatography (HPLC).This is done by taking 100 ml of 70% ethanol and adding it to 10 grams of the sample. Then heat the mixture with stirring for two hours, then the hot solution is filtered and the solvent is evaporated to dryness to obtain the precipitate that is considered a flavonoid[16].

Diagnosis of flavonoids using a device: HPLC

High-performance liquid chromatography (HPLC) was performed on a SYKAMN HPLC system (Germany) equipped with a C18-ODS column (5 µm, 6 mm.250 × 4). Samples (100 µm) were injected into the system. The mobile phase consisted of acetonitrile 95% + Triflouroacetic acid 01.0% (solvent A) and acetonitrile 5% + Triflouroacetic acid 01.0% (solvent B) at min/1 mL. The graduation program was as follows:10%A from 0-5 min; %25 A from 5-7 min; 40% A from 7-13 min; Then return to the initial conditions. Detection of phenolic compounds was performed using a UV-visible detector at 278 nm[17].

Induction of Myocardial infarction:

Myocardial infarction was induced in 6 groups of experimental animals using Triton Then the animals were left for 72 hours, after which the treatment phase began during the experiment period, which lasted 21 days, according to the following totals:

The first group, G1: (Control group) was given the standard diet and water only for 21 days.

The second group G2: (Triton X-100) was given 100 mg/kg of Triton X-100.

The third group: G3 (bay leaf flavonoids) was given 25 mg/kg bay leaf flavonoids.

The fourth group, G4: (Bay leaf flavonoids) was given 50 mg/kg of bay leaf flavonoids.

The fifth group, G5: (Bay leaf flavonoids) was given 100 mg/kg of bay leaf flavonoids.

The ninth group G6: (simvastatin) was given 5.0 mg/kg and simvastatin was given.

The basic principle for estimating the prolidase enzyme

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa plate provided in this kit is pre-coated with a PEPD-specific antibody. Standards or samples are added to appropriate naked Microelisa wells and conjugated with the specific antibody. A horseradish peroxidase (HRP)-conjugated antibody specific for PEPD is then added to each Microelisa-stripped well and incubated. Free components are washed off. TMB substrate solution is added to each well. Only those wells containing anti-PEPD and HRP-conjugated antibodies will appear blue and then turn yellow after addition of stop solution.

Determination of Sodium concentration in blood serum

The concentration of sodium in blood serum was estimated based on the colorimetric enzymatic method and using an analysis kit prepared by the Italian company Giesse Diagnostics [18].The level of sodium ions in blood serum was estimated based on the colorimetric method using a ready-made analysis kit from the German company Human.

Basic principle

This method is based on the fact that sodium is precipitated in the presence of (Mg-uranyl acetate) and the main uranyl ions form a yellow-brown complex suspension with thioglycolic acid. The difference between the standard solution and the analysis determines the concentration of sodium and is measured spectrophotometrically using a spectrophotometer at a wavelength of 405 nm [19].

Solution used

60 ml precipitating solution	Uranyl acetate 19 mmol /l Magnesium acetate 140 mmol/l
60 ml colour reagent	Ammanium thioglycolate 550 mmol/l
2 ml Standard	Ammonia 550 mmol/l Sodium

Procedure

Three tubes were prepared

	Blank	**Standard (ml)**	**Sample (ml)**
Standard	-	50	-
Sample	-	-	50
Precipitating solution	-	3000	3000

I mixed the tubes well and left for 5 minutes and mixed well for 30 seconds. It was left for 30 minutes, then placed in a centrifuge at the highest speed for 10 minutes. Then the following materials were added to it:

	Reagent Blank	**Standard (ml)**	**Sample (ml)**
Precipitating solution	50	-	-
Clear supernatant	-	50	50
Colour reagent	3000	3000	3000

The tubes were mixed well, and after 30 minutes, the color intensity of the Reagent Blank was measured, then the sample, at a wavelength of 405 nm.

Calculation

Sodium in blood serum was calculated according to the following equation:

$$\text{Sample concentration} = \frac{\text{ARB-A sample}}{\text{A std ARB-}}$$

A = Absorbance

RB = Reagent blank

Std = Standard

Analysis Statistical

"The results were analyzed statistically using the program (SAS, 2001) and according to one-way analysis of variance, and the arithmetic means of the coefficients were tested using the Duncan multiple range test at a significance level (0.05) to determine the significant differences between the groups [20].

Results and discussion

Analysis of the active components of the laurel plant via HPLC

The results of the analysis using HPLC technology recorded the concentrations of the active ingredients, including flavonoids. Table (1) shows these concentrations. We find that the concentration of Rutin, Kaempferol, and Qurcetine was the highest, reaching 41.58, 33.05, and 32.58 mg/ml, respectively, followed by Gallic acid, Luteolin, and Rosemaric acid. Apigenin reached 30.25, 25.64, 16.55, and 16.25 mg/ml, respectively. These results agree with [21] high concentrations of flavonoids, which reached 12%.

Table (1) shows the concentrations of each of the flavonoids of bay leaf

Name (ppm)	**laurel plant**
Kaempferol	33.05
Luteolin	25.64
Qurcetine	32.58
Rutin	41.58
Apigenin	16.25
Gallic acid	30.25
Rosemaric acid	16.55

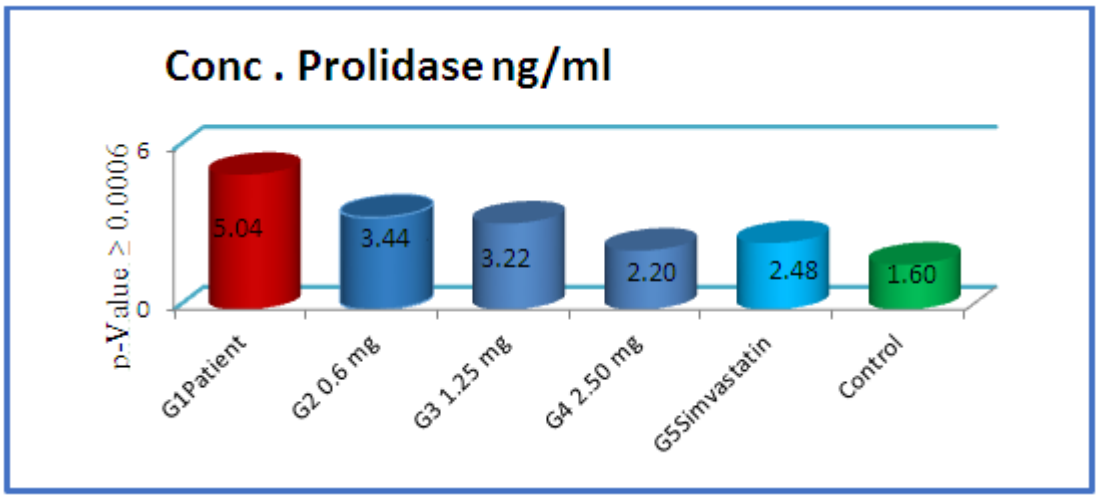
Determination of prolidase enzyme

Table (2)and scheme(1). shows the levels of the prolidose enzyme in the treatment with myocardial infarction and the rest of the experimental treatments. We find that the enzyme increased (5,045 ng/ml) compared to the normal control (1.603). This result is consistent with (Latfu Askin and Okan Tanriverdi) [22] .who indicated an increase in the level of Prolidase after cardiac injury because it plays a vital role in restoring collagen metabolism and reducing platelets attached to the arteries. Collagen plays an important role in reducing stress, adenoid fibrillation, and vascular dilation diseases. Dosing animals led to increased concentrations of bay leaf. (0.6, 1.25, 2.50 mg) led to a decrease in the level of Prolidase to (3.445, 3.223, 2.207 ng/ml) for the mentioned doses, respectively, and this result agrees with (Graziano Lolli et al) [23].Who indicated that flavonoids have high effectiveness against oxidative stress and remove free radicals such as RNS and ROS, thus inhibiting the oxidation of LPL-C and restoring their vitality to cardiac cells, thereby assisting the prolidase enzyme in its activity,

reducing the need to increase its secretion. As for the use of simvastatin, it has led to a reduction in Prolidase concentration to the level of (2,487) and this result agrees with (Arif Sunner; Abdullah, N; Mustafa P and Hakan K) [24].who found an increase in the enzyme in patients suffering from microvascular injury and high blood pressure, but treatment with statin led to a reduction in the level of the enzyme, and this indicates the animal's response to treatment, knowing that the results of this treatment were close to the concentrations. Which we obtained after dosing with rosemary plants.

Table 2. Effect of prolidase enzyme

P-Value	Control	Simvastatin drug	G4 2.50 mg	G3 1.25 mg	G2 0.6 mg	Patient	**group**
0.0006**	1.603 0.064± E	2.487 0.095± Cd	2.207 0.067± D	3.223 0.375± B	3.445 ±0.247 B	5.045 0.658± A	**Prolidase ng/ml**



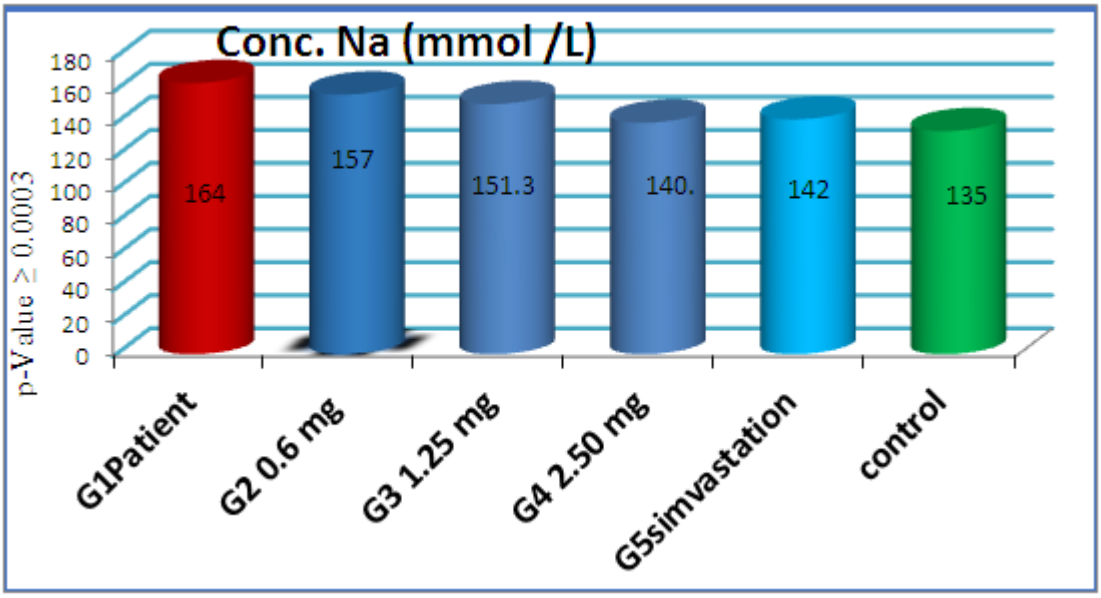
Scheme 1. Determination of prolidase enzyme

Estimation of Sodium level

The results shown in Table (3) and scheme(2). showed an increase in the level of sodium in the infected sample, reaching (164.00 mmol/L). These results did not agree with Talebi, S. Ghobadi, F and Cacacho.A.) [25] who found a decrease in the level of sodium hyponatremia in patients with myocardial infarction but agreed with (Soubhag, P; Radhika, M. and Pushpalata) [26] who indicated a decrease in the level of sodium In non-surviving patients, as for patients who survived myocardial infarction (AMI), the level of sodium increased, which was attributed to the fact that AMI patients suffer from hypertensive blood pressure and the effect of Aldosterone, Vasopressin, and Epinephrine on the regulation of blood electrolytes.

Table 3. Effect of Sodium level

P-Value	Control	Simvastatin drug	G4 2.50 mg	G3 1.25 mg	G2 0.6 mg	Patient	**group**
0.0006 **	135.00 ± 7.00 e	142.00 ± 2.00 c	140.00 ±2.00 d	151.33 ± 2.08 c	157.00 ± 4.24 b	164.00 2.83± a	**Na mmol/l**



Scheme 2. Determination of Sodium level

Conclusions

The study showed a significant increase in prolidase enzyme with a decrease in its concentration compared to the control group. When the sodium concentration was measured, a significant increase was observed compared to the control group.

reference

1. Özcan, M., & Chalchat, J. C. (2005). Effect of different locations on the chemical composition of essential oils of laurel (Laurus nobilis L.) leaves growing wild in Turkey. *Journal of Medicinal food*, *8*(3), 408-411.
2. Konovalov, D. A., & Alieva, N. M. (2019). Pheno lic compounds of Laurus no bilis. *Фармация и фармакология*, *7*(5 (eng)), 244-259.
3. Dammak, I., Hamdi, Z., El Euch, S. K., Zemni, H., Mliki, A., Hassouna, M., & Lasram, S. (2019). Evaluation of antifungal and anti-ochratoxigenic activities of Salvia officinalis, Lavandula dentata and Laurus nobilis essential oils and a major monoterpene constituent 1, 8-cineole against Aspergillus carbonarius. *Industrial Crops and Products*, *128*, 85-93.
4. Kaurinovic, B., Popovic, M., & Vlaisavljevic, S. (2010). In vitro and in vivo effects of Laurus nobilis L. leaf extracts. *Molecules*, *15*(5), 3378-3390.
5. Reed, G. W., Rossi, J. E., & Cannon, C. P. (2017). Acute myocardial infarction. *The Lancet*, *389*(10065), 197-210.
6. Lavie, C. J., De Schutter, A., Parto, P., Jahangir, E., Kokkinos, P., Ortega, F. B., ... & Milani, R. V. (2016). Obesity and prevalence of cardiovascular diseases and prognosis—the obesity paradox updated. *Progress in cardiovascular diseases*, *58*(5), 537-547.
7. Shiekh, P. A., Mohammed, S. A., Gupta, S., Das, A., Meghwani, H., Maulik, S. K., ... & Kumar, A. (2022). Oxygen releasing and antioxidant breathing cardiac patch delivering exosomes promotes heart repair after myocardial infarction. *Chemical Engineering Journal*, *428*, 132490.

8. Capotosto, L., Massoni, F., De Sio, S., Ricci, S., & Vitarelli, A. (2018). Early diagnosis of cardiovascular diseases in workers: role of standard and advanced echocardiography. *BioMed Research International*, *2018*.

9. Butbul Aviel, Y., Mandel, H., Avitan Hersh, E., Bergman, R., Adiv, O. E., Luder, A., & Brik, R. (2012). Prolidase deficiency associated with systemic lupus erythematosus (SLE): single site experience and literature review. *Pediatric Rheumatology*, *10*, 1-7.

10. Soran, N., Altindag, O., Aksoy, N., Cakır, H., Taşkın, A., Soran, M., & Işıkan, E. (2013). The association of serum prolidase activity with developmental dysplasia of the hip. *Rheumatology International*, *33*, 1939-1942.

11. Marotte, H., Gineyts, E., & Miossec, P. (2009). Prolidase deficiency: a rare aetiology of arthritis. *Joint Bone Spine*, *77*(1), 88-89.

12. Zavalishina, S. Y., Karpov, V. Y., Yuryevna, A., Zagorodnikova, A. A. R., Alikhojin, R. R., & Voronova, N. N. Functional Mechanisms for Maintaining Posture in Humans during Ontogenesis. (2021). *Journal of Biochemical Technology*, *12*(1), 36-39.

13. Khonsary, S. A. (2017). Guyton and Hall: textbook of medical physiology. *Surgical neurology international*, *8*.

14. Frohlich ,E.D. and Jasmina,V.and Brenda, The Role of sodium in Hypertension is more complex than simply elerating pressure . (2004). Me.J. forcardio V.med .12:1-3.

15. Schakhmatova,E.I;Osipova,N.andNatochin,y,U.change in osmolality and blood serum ion concentration in pregnancy .(2000).Hum .physiol.J.26(1)92-95.

16. Khonsary, S. A. (2017). Guyton and Hall: textbook of medical physiology. *Surgical neurology international*, *8*.

17. Bishop, M. L. (2020). *Clinical Chemistry: Principles, Techniques, and Correlations, Enhanced Edition: Principles, Techniques, and Correlations*. Jones & Bartlett Learning.

18. Berry, M. N.; Mazzachi, R. D.; Pejakovic, M.; & Peake, M. J. (1988). Enzymatic determination of sodium in serum. *Clinical chemistry*. Vol.34, no.11, pp.2295-2298.

19. El-Ghany, A., Ramadan, A. H. M., Nagib, R. M., & Mahmoud, F. M. (2016). Performance of Hypolipidemic Activity of Persimmon Extracts on Triton-X Induced Hyperlipidemia in Male Rats. *Sciences*, *6*(03), 636-645.

20. Duncan, D. B. (1955). Multiple range and multiple F tests. *biometrics*, *11*(1), 1-42.

21. Škerget, M., Kotnik, P., Hadolin, M., Hraš, A. R., Simonič, M., & Knez, Ž. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food chemistry*, *89*(2), 191-198.

22. Latfu Askin and Okan Tanriverdi .(2021). Serum Prolidase Activity In Cardiovascular Disease. Research Gate.

23. Lolli, G., Cozza, G., Mazzorana, M., Tibaldi, E., Cesaro, L., Donella-Deana, A., ... & Pinna, L. A. (2012). Inhibition of protein kinase CK2 by flavonoids and tyrphostins. A structural insight. *Biochemistry*, *51*(31), 6097-6107.

24. Suner, A., Nurdag, A., Polat, M., Kaya, H., Koroglu, S., Acar, G., & Sezen, H. (2015). Evaluation of serum prolidase activity in patients with slow coronary flow. *Advances in Interventional Cardiology/Postępy w Kardiologii Interwencyjnej*, *11*(3), 206-211.

25. Talebi , S ; Ghobadi , F and Cacacho .A. (2016). Looking at diabetic ketoacidosis through electrocar diagram Amer. J. of Medicine. 34 (2) : 263-265.

26. Soubhag , P ; Radhika , M. and Pushpalata .(2019) Study of serum sodium , potassium , calcium in patients with myocardial infarction . annals of inter medical and dental res. 5 : (5).