



The Beneficial Influence of Rosemary Aqueous Extract on The treatment of Oxidative Stress-Induced Myocardial Ischemia in Rats



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Abstract

Ischemia “stroke” may occur in various combinations of clinical illnesses. The effects of such an Ischemic represent a serious problem. Oxidative stress has been shown to act as one of the main causes of Myocardial Ischemia- Reperfusion Injury (IR). Reperfusion injury is defined as the action of returning the flow of blood to an organ or tissue after a period of stroke or myocardial infarction (MI). *R. Officinalis* (Rosemary) has a diverse therapeutic against many illnesses. Further, recent evidence also gives *R. Officinalis* a major role in protecting diverse organs from harmful oxidative stress. So, *R. Officinalis* seems like a good protect to the myocardial against the IR Injury. The study included the components of essential oils of the rosemary leaves in the watery extract and determine whether rosemary protects form Ischemia-Reperfusion induced myocardial ischemia. We divided forty-five Wister rats into three groups Group 1 as the control group, Group 2 underwent surgery without extract therapy, and Group 3 with extract therapy. Wister rats in Group 2, and Group 3 underwent myocardial ischemia for 45 min followed by 90 min period of Reperfusion. Animals in group 3 received rosemary (1 mL/g) for 14 days also, before Ischemia, and before Reperfusion. We found that the extract comprises from many essential oils. The levels of cardiac markers (AST, ALT, and LDH) and Oxidative stress markers (NO, and LPO) in group 3 a significantly decreased compared to group 2. Based on the results, the *R. Officinalis* treatment lessens the effect of Ischemia-Reperfusion on the rat’s heart, including the induce myocardial infarction.

Key words: *R. Officinalis*; Ischemia-Reperfusion; Myocardial infarction

1. Introduction

Ischemic heart disease is one of the main causes of disability and death worldwide[1,2]. The most common reason for myocardial infarction (MI) is coronary artery disease (CAD)[3]. CAD accounts for almost 50% of all myocardial ischemia [4, 5]. Improving the blood flow (Reperfusion) is the ultimate goal of the therapy of Ischemia. The successful rate of Reperfusion is 50 to 70% after an injury occurs normally[6]. Reperfusion’s type of treatment depends on the type of ischemia. It can be achieved either by a procedure called an endovascular (physical removal of a large blood clot), or a mechanical thrombectomy. A wire-cage device called a stent retriever can be used in order to remove the large blood clot in addition to the thrombus

disruption using stents. Intravenous medical treatment of recombinant tissue plasminogen activator is the only FDA-approved drug for acute ischemic heart therapy. The tissue plasminogen activator thrombolysis treatment is 4.5 hours after the ischemia[7]. Patients in the latest years underwent many clinical trials for embolectomy surgery with stent retrievers. The results were beneficial for the patients and led to the use of this type of treatment thereon[8]. Oxidative stress is increased after ischemia-reperfusion, as a result of reactive oxygen species (ROS) generated extra especially from phagocytes as well as endothelium causing damage to all component of the heart muscle cells [9]. On the other hand, Reperfusion injury occurs as a result of oxygen restoration. This happens very rapidly and it

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leads to harmful influences on the myocardial. The preceding is worse than certain types of MI. That was proven by experiential studies and clinical guide damage effect of Reperfusion injury after MI[10].

Since herbs contain a high concentration of antioxidant properties, therefore they have always been used in the treatment of degenerative diseases like cardiovascular, neurological, and cancer^[11, 12]. *R.Officinalis* is a significant medicinal herb from the Lamiaceae family native from the Mediterranean area and planted for a long time in Iraq[13].

It is well known that common medical therapy has side effects, relating to its long-term use, which could be very costly for the patient. Therefore we tend to lean more towards herbs and alternative treatments[14]. We found that *R.Officinalis* contained antioxidant properties and is used in the field of medicine. In addition, *R.Officinalis* has anti-tumorigenic, anti-inflammatory, antidiabetic, anticancer, antiproliferative, and neuroprotective properties^[15-18].

2. MATERIALS AND METHODS

2.1. Ethical statements

This study was done after the approval of the AL-Mustansiriyah university ethics committee.

2.2. Plant material

R. officinalis was obtained from the local markets in Baghdad Iraq.

2.3. Extraction of essential oils

R. officinalis oil extract was obtained by mixing *R. officinalis* leaves with distilled water, putting the mixture in a Clevenger apparatus, and leaving it for 3 hours under a temperature of 100° C, and through the process of steam distillation, the mixture of oil and water was collected in an opaque bottle. Finally, the oil sample was separated from the water, by adding anhydrous Na₂SO₄ and stored in vials, to GC-MS analysis.

factor- α (TNF- α), and interleukin-1 β (IL-1 β) levels were evaluated by immunoassay kits (Endogen, Woburn, MA, USA).

2.4. Experimental Design:

Forty-five male Wister rats with weight 215 ± 15 and 5 to 6 weeks of age, were enrolled in this study, were kept in the standard laboratory. Then the rats were divided into 3 groups (n = 15 in each group), which were fed a standard diet. As follows: the first group marked as the control group 1, the second group was I/R group (underwent surgery without extract therapy) group 2, whereas the third group was I/R with Intraperitoneal injection of (1mL. g⁻¹) dose of Watery extracts *R.officinalis* (group 3).

2.5. Surgical procedure:

Experimental rats were not fed for 24 hr before the surgery. The male Wister rats subjected to 45min myocardial Ischemia by occlusion of the left anterior descending (LAD) coronary artery followed by 90 min Reperfusion. The control group (group 1) underwent the same surgical procedures from anaesthesia, open chest at the left 4th. Intercostals space and separation of the arteries [without blocking the arteries]. All animals were carried out underwent general anaesthesia [all rats received 70 mg.kg⁻¹ pentobarbital sodium]. During the surgical procedures, we kept the rats' body at a normal temperature (98.6 ° F) by a warm board. Finally, blood samples were collected via an abdominal aortic artery [19].

2.6. Biochemical estimations:

Biochemical parameters were measured by taking blood samples from the animals.

1) Cardiac markers: aspartate transaminase (AST) [20], alanine transaminase (ALT) [20], and lactate dehydrogenase (LDH) were evaluated to determine activity of heart muscles, by kits {aeroset® Abbott Laboratories, Chicago, IL}.

2) Oxidative stress markers: we used the left-hemisphere to evaluated nitric oxide (NO) of total nitrate [21], and (Thiobarbituric Acid Reactive Substances) was used to detect lipid peroxidation (LPO) [22] . Interleukin-8 (IL-8), tumor necrosis

All analyses were done with the SPSS-24 software (version 24). The differences were be considered significant at $p < 0.05$. All values are expressed as mean \pm SD.

2.7. Histopathological studies

An autopsy was performed in all-male Wister rats in the group 2, and 3, and their hearts were removed and preserved in 10 percent neutral formalin. After that, hearts were embedded in a wax block to be sectioned 4 microns (μm) and stained for the histopathological estimation using natural black 1 and Eosin stains. Finally, histopathological estimation was carried out using a light microscope.

3. RESULTS

Various essential oils were found in the aqueous extract of the plant which was listed in table (1). Reperfusion injury caused a significant elevation in ROS production for group 2 which led to inflict heart

muscles as indicated by significant increases for ALT, AST, and LDH in the blood compared to group 1 (control group). In contrast; the levels of AST, ALT, and LDH were reduced in group 3 given R. Officinalis extract compared to group 2 as shown in table(2).

Rising LPO and NO indicate oxidative stress in group 2 compared to group 1 (control group). Whilst, the levels LPO and NO were reduced in group 3 injected with R. Officinalis extract compared to group 2 as shown in table (3).

The results showed in table 4 that remedy group rats (group 3) exhibit a significant decrease ($p < 0.01$) in IL-8, TNF- α , and IL-1 β as compared to the (Group 2).

Table1. The percentage composition of essential oils detected in the aqueous extract

NO.	compound	Kohat's index	Percentage (%)
1	α -pinene	932	50.6
2	camphene	947	5.4
3	β -pinen	978	1.9
4	α -Terpinene	1025	0.4
5	α -Terpiolene	1072	0.5
6	Limonene	1092	1.9
7	cineole	1108	24.2
8	β -myrcene	1149	0.9
9	γ -terpinene	1167	0.6
10	Linalool	1186	1.4
11	Camphor	1197	3.7
12	Borneol	1208	1.6
13	α -Terpineol	1284	0.9
14	Verbenone	1418	0.6
15	Bornyl acetate	1422	1.7
16	β -Caryophyllene	1426	2.8
17	α -Caryophyllene	1459	0.4

Table 2. Influence of *R. officinalis* extract on enzymes heart in the Wistar rat model.

Bio-markers	Group 1	Group 2	Group 3	P value
	Control group			
ALT (IU/L)	31.6 ± 3.18	195.9 ± 19.1	138.29 ± 12.72	< 0.001
AST (IU/L)	127.11 ± 9.18	798 ± 27.54	576 ± 17.36	< 0.001
LDH (IU/L)	298 ± 28.6	1144.9 ± 85.3	862 ± 84.3	<0.001

Abbreviation; AST: aspartate transaminase, ALT: alanine transaminase, LDH: lactate dehydrogenase. P<0.05.

Table 3. Influence of *R. officinalis* extract on LPO and NO levels in the Wistar rat modal.

Bio-markers	Group 1 (n=15)	Group 2	Group 3	P value
	Control group			
		(n=15)	(n=15)	
NO (umol/L)	254 ± 14.35	436 ± 23.8	321 ± 18.2	P < 0.001
LPO(nmol/mg)	2.7 ± 0.21	8.26 ± 0.64	6.49 ± 0.5	P < 0.001

Abbreviation; NO: nitric oxide, LPO: lipid peroxidation. P<0.05

Tables 4. Influence of *R. Officinalis* on IL-8, TNF- α , IL-1 β levels in Wister rat model.

Bio-markers	Group 1 (n=15)	Group 2	Group 3	P value
	Control group			
		(n=15)	(n=15)	
IL-8 (ug/mL)	0.63 ± 0.12	1.92 ± 0.36	1.35 ± 0.25	P< 0.01
TNF- α (ng/L)	22.19 ± 12.06	46.42±13.16	38.23± 11.07	P< 0.01
IL-1 β (pg/ml)	115.7 ± 11.4	339.5 ± 32.6	235.8 ± 22.7	P< 0.01

Abbreviation; IL-8: Interleukin-8, TNF- α : tumor necrosis factor- α , IL-1 β : interleukin-1 β . P<0.05.

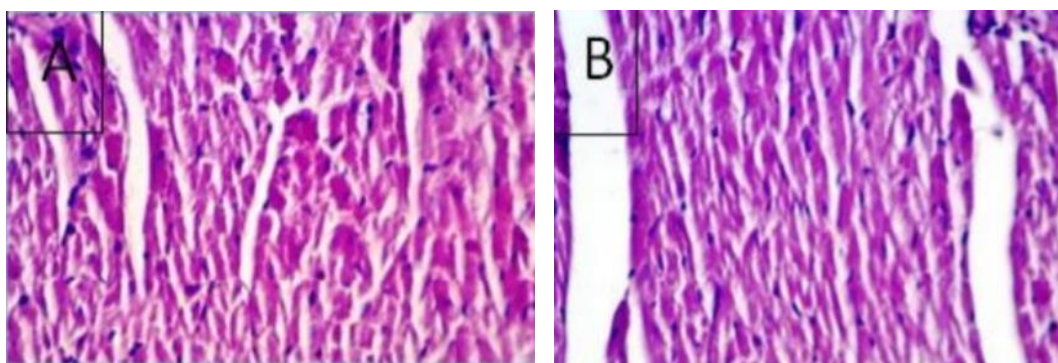


Fig. 1. photomicrographs of Wister Heart section – A) Group 3: treated with *R. Officinalis*, cardiac muscle structure shows few apoptotic cells, B) Group 2: after 90 min I/R, cardiac muscle structure shows a lot of apoptotic cells.

4. Discussion:

It was determined that the qualitative chemical analyses of the aqueous extracts for the composition of essential oils were similar to other studies that were reported [23, 24]. In our experiments, we found that the ALT, AST, and LDH were significantly lower in Group 3 compared with Group 2. This result is in agreement with the findings of Karataş et al.[25], and Almakhatreh et al.[26]. *R. Officinalis* extract greatly lowered the blood-brain barrier permeability disruption and injuries, moreover, it lessens the effect of the myocardial infarction and neurological deficit scores after focal Ischemia-Reperfusion. *R. Officinalis* leads to the protection of rats from myocardial injuries. It also lowers the cognitive deficit which is caused by ischemia.

Oxidative stress is the main factor that causes ischemic injuries and results in coronary artery disruption and cell apoptosis [27-29]. In addition, we found that the extract lowers LPO and NO in Group 3 than in Group 2. This is in agreement with the findings of Seyedemadi et al.[30], and Posadas et al.[31] they were found the extract lower free radical activities in rat serum, brain, liver, kidney, and heart tissues. After those experiments, it is clear that it has strong antioxidant properties[16], even more than the phenolic compounds [32].

The data indicate that the use of *R. Officinalis* extracts caused an improvement of diastolic function, in addition to reduced hypertrophy after MI; it is successfully protected against oxidative stress which

in turn leads to cardiac hypertrophy. Therefore, *R. Officinalis* adverse cardiac remodeling through reducing hypertension, myocardial infarction, improving energy metabolism, and blood supply to the heart [17], [33-35]. Inflammatory cytokines have been playing a protruded role in fibrosis, and cell death[36- 38]. Our results appeared that the IL-8, TNF- α , and IL-1 β were significantly lower in Group 3 compared to Group 2. The same results were obtained by Mengoni et al.[39]. They were observed that *R. Officinalis* extract is an anti-inflammation through reducing pro-inflammatory enzymes, white blood cell activities, and mediators like IL-1 β , TNF- α , and NO. and Yu et al.[40], he explained the work *R. Officinalis* extract by inhibiting or reduce phosphorylation of MAPKs, also blocking receptor activator of NF κ B, thus lowering expression of NOS and Cyclooxygenase-2.

5. Conclusion

In conclusion, the aqueous extract of *R. Officinalis* protects rats' from ischemia-reperfusion injury. It seems to be useful in reducing ischemia-reperfusion outcomes, and related other diseases.

6. Conflicts of interest

We haven't any conflicts of interest with any agency.

7. Acknowledgments

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