

The protective and therapeutic effect of alcoholic extract of rosemary officinalis against degeneration effect of over dose of paracetamol on the heart muscle of rats.

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Key Words: Paracetamol, Rosmarinus officinalis, Cardio toxicity, Rat

Abstract

The experiment were performed in present study to estimate the protective and the therapeutic role of ethanolic extract of rosemary (*Rosmarinus officinalis*) leaves against acute destructive changes in rats due to high dose of paracetamol, 1ml/ 1000mg/ kg that led to a particular aspect of heart necrosis, congestion, vacuolation of heart muscle cells, and infiltration of inflammatory cells. Leaves and stem of (*Rosemary officinalis*) were collected, identified, dried, powdered and extracted by using 90% ethanol in soxhlet apparatus. Twenty healthy albino mature male rat's *Rattus norvegicus* were used in this study were divided to four groups, including group1 (control group) group 2 were given over-dose of paracetamol for 3 weeks, group 3 were given combination consist from high dose of paracetamol and alcoholic extract of (*Rosemary officinalis*) for 6 weeks, and group 4 were given rosemary extract for 3 weeks after it was given over-dose of paracetamol for 3 weeks. The histopathological result showed that the alcoholic extract of rosemary officinalis have the protection effect when used in combination with high dose of paracetamol that clear in microscopic appearance of the heart muscle in group 3, while the histopathological result of group 4 revealed the therapeutic effect to the alcoholic extract of rosemary officinalis and the microscopic appearance showed the intact heart muscle. The present study revealed the productive and treated role to alcoholic extract of rosemary officinalis on heart muscle after long term of overdose of paracetamol that showed degeneration and vacuolation in muscle fiber addition that infiltration of inflammatory cell, the extract of this plant resolve the pad tissue changes on the heart tissue especially in group 4 that appear intact heart muscle and in group 3 showed reduce the sign of the overdose.

التأثير الوقائي والعلاجي لمستخلص اكليل الجبل الكحولي ضد التأثيرات الانحلالية للجرعة العالية للباراسيتامول في قلب الجرذان

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الخلاصة

لقد أجريت هذه الدراسة لتقييم فاعلية المستخلص الكحولي لنبات إكليل الجبل (الأوراق والساق) العلاجية وللحماية ضد التأثير السمي للباراسيتامول بجرع عالية 1000 ملغم / كغم من وزن الجسم على القلب في ذكور الجرذان البيضاء. بعد جمع أوراق وسيقان نبات إكليل الجبل تم توصيفها وتجفيفها وطحنها و تم استخلاصها بواسطة الكحول المثلبي بتركيز 90-95% باستخدام جهاز السكسوليت. تم استخدام 20 جرذ مختبري قسمت الى اربعة مجاميع، المجموعة الاولى مجموعة سيطرة اعطيت ماء وعلف طوال فترة التجربة والمجموعة الثانية اعطيت جرعة عالية من الباراسيتامول 1000 ملغم / كغم من وزن الجسم وعن طريق الأنبوب المعدي ولمدة 3 اسابيع، والمجموعة الثالثة جرعت بنفس الجرعة للباراسيتامول بعد تجريعها بالمستخلص الكحولي لنبات إكليل الجبل 250 ملغم / كغم من وزن الجسم، بينما المجموعة الرابعة تم تجريعها بالمستخلص الكحولي لنبات إكليل الجبل 250 ملغم / كغم من وزن الجسم عن طريق الأنبوب المعدي لمدة 3 اسابيع بعد ان اعطيت جرعات عالية من الباراسيتامول ولمدة 3 اسابيع. اظهرت نتائج الفحص النسيجي للمجموعة 3 ان هناك تغير كبير في المظهر النسيجي اذ قلت اعداد الخلايا الالتهابية والتفجي داخل خلايا عضل القلب بالإضافة الى قلة الاحتقان في الاوعية الدموية داخل القلب، بينما اظهرت النتائج النسيجية للمجموعة 4 ان للمستخلص دور علاجي فعال ضد الانتكاس وترشح الخلايا الالتهابية والاحتقان وبينت سلامة نسيج القلب من مظاهر السمية للجرعة العالية للباراسيتامول في كل من المجموعة 4 التي اظهرت نسيج سليم والمجموعة 3 التي اظهر نسيجها تقليل واضح للآثار السلبية للجرعة العالية للباراسيتامول.

1. Introduction

Rosmarinus officinalis is a considered one of the mint family Lamiaceae, along with many other herbs, like the basil, thyme, oregano, and lavender. In addition to good taste rosemary in cuisine, also a useful exporter of iron, vitamin B-6, and calcium. Habitually rosemary is the one herbs which have a wide range uses in popular medicine or beauty products, also used as seasoning for the foods. [1]

In the past, herbal medicine has taken an active and important role in alternative and popular medicine, which is used to treat many various diseases. [2] "*Rosmarinus officinalis*", have a variety of anti-tumor, antioxidant, and anti-inflammatory activities and elevate the immune system and betterment blood circulation. Many resercher have discern the "*Rosmarinus officinalis*" extract is very wealthy in anti-oxidant that play an important role in equilibrium and removal the harmful of the free radical. Rosemary leaves are used in medicine folk as an analgesic, antispasmodic, diuretic and antiepileptic agent [3][4]. A large number of natural products of herbs that are found in food and that give positive and protective effects of the body. In affected organs within the body one or more of the following actions lead to devlry of the tissue: (a) free radicals slink (b) cellular antioxidants altitude (c) boost for the bone marrow

recovery (d) regeneration of extra hematological tissue, Medicinal and herbal plants that proposes the main mechanisms for the protection. [5] The phenolic compounds and rosemarnic acid are considered to be the most important components of rosemary, which are characterized by their anti-free radicals effectiveness, these constituents is well absorbed from gastrointestinal tract and from the skin. [6]

There are many vital activities of leaves of rosemary, such as resistance to all cancers, infections, bacteria, as well as its antioxidant effectiveness due to the presence of caffeic acid and its derivatives. The vital activities of rosemary extract are identical with known anti-oxidants compounds, like carnosol, arnosic acid, rosemarenic acid, butylated hydroxyanisole, butylated hydroxytoluene and ursolic acid, without confederate hazard to carcinogenic or cyto-toxicity of the artificial antioxidants. [7, 8, 9] One of the most common non-steroidal anti-inflammatory drugs is paracetamol. The heart muscle after long term and high dose of paracetamol suffering from many subtle histological changes such as gaps in the heart muscle and myocardiac atrophy, as well as fibrosis in the outer wall of the heart. [10, 11] The histological structure of the heart and vessels tissue from mice treated with paracetamol showed moderate vascular congestion and inflammatory alteration like the myocytes coagulation and cardio-toxicity. [12, 13] The higher doses of paracetamol and for long periods lead to many risks of reverse response, typical for selective inhibition of COX-2, such as hypertension, heart infarction (necrosis) or renal failure. [14] The aim of this research is to identify and assess the preventive and therapeutic role of the rosemary extract by ethanol alcohol on heart muscle versus the long term of overdose of paracetamol.

2 Materials and method

2.1 Experiment Animals

Twenty healthy albino mature male rat's *Rattus norvegicus* were used in this study. These animals weighing 350-450 gm $\pm$ 5g. The animals were obtained from the Animal house of the pharmacy collage University of Kerbela. Housing conditions were maintained at 20-25C in air-conditioned room, air of the room was changed continuously by using ventilation vacuum, while the light/ dark cycle was 14/10 in housing place. The litter of the cages was changed weekly. They were given a rodent diet supplement with a Vitamin C and supplied with water ad libitum. The animals were left in normal conditions for two weeks for acclimatization in animal house.

2.2 Experimental design

Twenty adult male rats were divided equally into four groups (5rat/group) as the following

Group 1: given distal water orally (control).

Group 2: administered paracetamol 1ml 1000 Mg/Kg, by gavage needle.

Group 3: administered combination paracetamol 1ml 1000mg/Kg, and 250 Mg/Kg alcoholic extract of rosemary by gavage needle.

Group 4: administered 250 Mg/Kg alcoholic extract of rosemary for 3weeks after administered by paracetamol 1ml 1000 Mg/Kg, for 3 weeks by gavage needle.

2.3 Experiment Ethanolic Leaves Extract

Mellow Rosemary officials' leaves and stem were collected from medical garden in pharmacy collage (Kerbela) from May to June. The leaves and stem were washed in fresh water to remove adhering dust and then dried under shade. The dried leaves and stem were grinding by a laboratory blender. The plant classification was done Dr. Ibraheim Saleh Abbas of the Pharmacognosy department, pharmacy faculty, Al-Mustansiriya University.

2.4 Extraction of plant

Extraction was done by using Alcoholic solvent extraction of the ethanol (90-95%) for the Rosemary officials' leaves and stem. Alcohol extraction is an effective method of isolating active substances by using the Soxhlet apparatus according to method described by [15].

2.5 Experimental of Histopathology

For histopathological study the rats were anesthetized by high dose to sacrifice them. Immediately, after sacrifice, the heart was removed and preserved it in 10% neutral formalin buffer solution. The histological sections were stained routinely by hematoxyline and eosin. Several tissue sections (1cm) were prepared according to [16].

The light microscope was used for examination the slide to revel the histopathological changed.

Result

The histopathological study for animal treated with high dose of paracetamol followed by administration with rosemary extract showed the following:

Group 1 :These group showed the normal histological structure for the heart muscle (Figure 1, 2)

Group 2: The cardiac muscle in these group suffering from degeneration and necrosis, and there is markedly separation between the muscle fiber and vacuolation in myocardial cells, also there are presence of globular hemorrhage with infiltration of inflammatory cells (Figure 3, 4, 5)

Group 3: The cardiac muscle showed mild vacuolation in muscle cells fiber, decreased the degeneration and little infiltration of inflammatory cells, also there is a fibrosis in pericardial region (Figure 6, 7).

Group 4 : In this group the heart muscle appears like normal and intact cardiac muscle with moderated congestion of vessel. (Figure 8, 9)

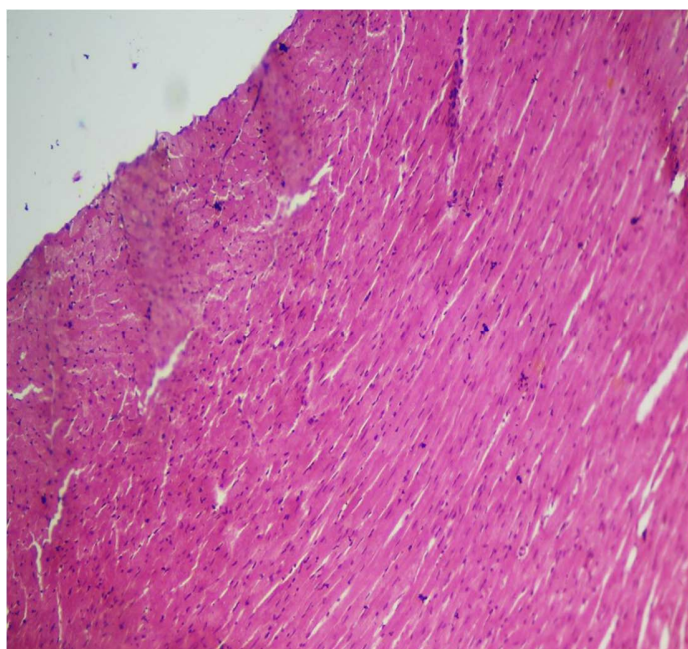


Figure 1 showed the control group normal microscopic appearance and intact cardiac muscle H&E X400

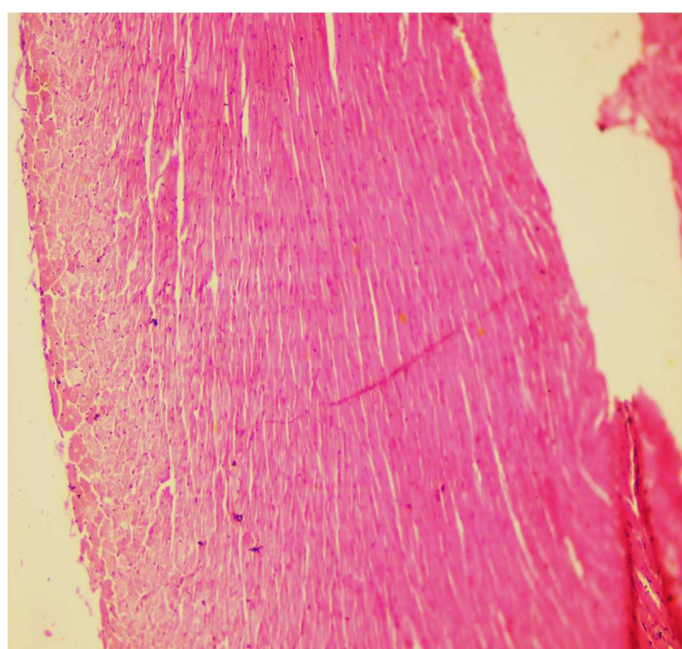


Figure 2 showed the control group normal microscopic appearance and intact cardiac muscle H&E X400

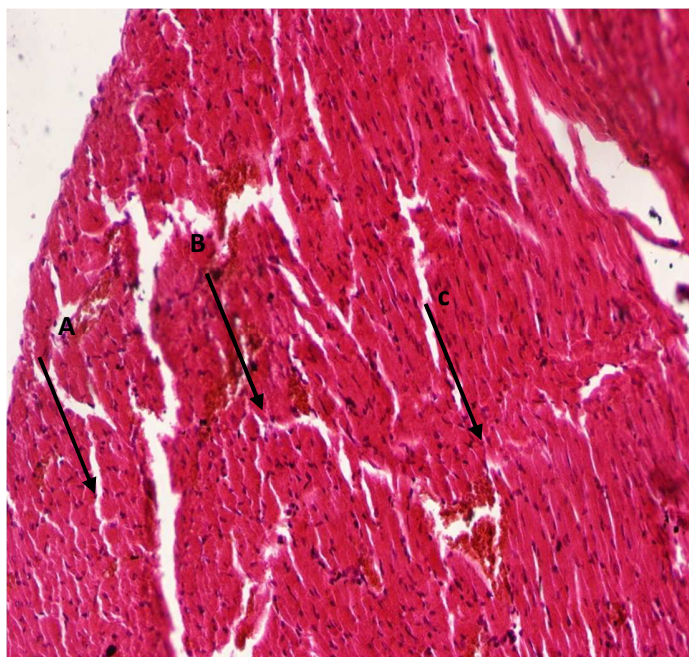


Figure 3 heart of high dose of paracetamol showed the A) degeneration B) inflammatory cells infiltration C) congestion H&E X400

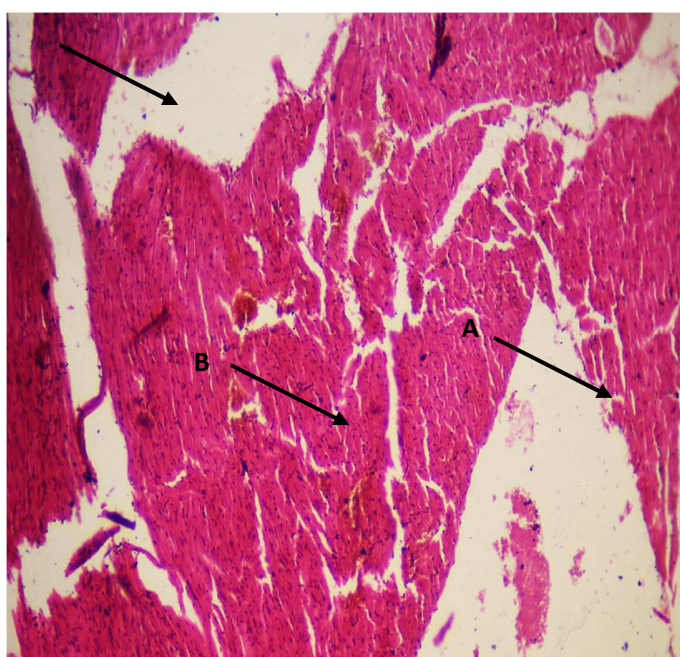


Figure 4 heart of high dose of paracetamol showed the A) separation muscle fibers B) congestion C) inflammatory cells infiltration H&E X400

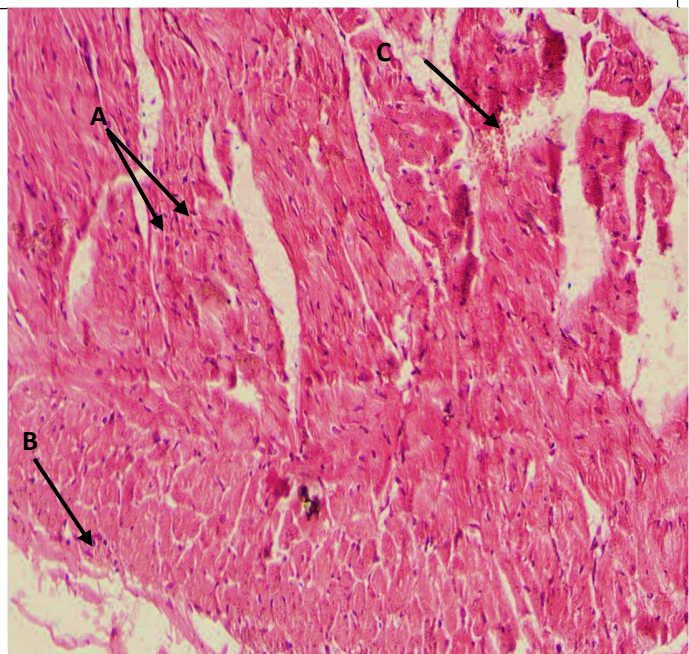


Figure 5 heart of high dose of paracetamol revealed A) atrophied muscle cells B) fibrosis C) hemorrhage H&E X600

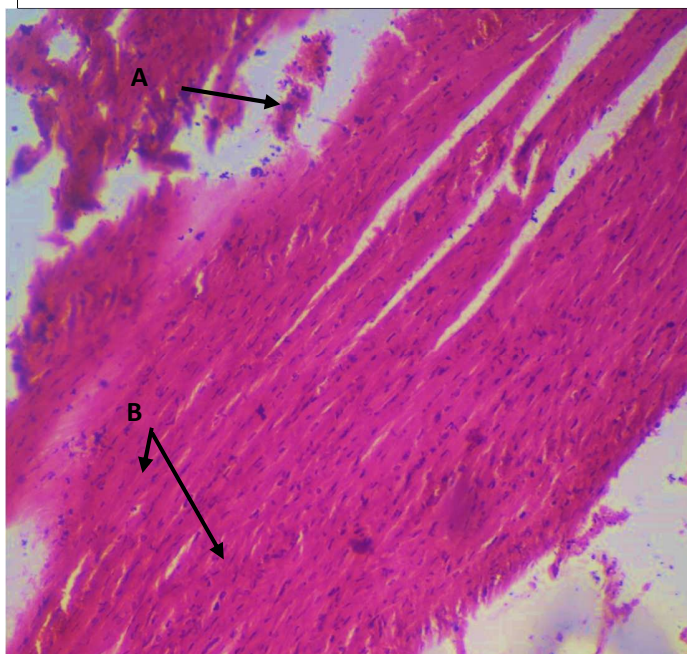


Figure 6 heart of high dose of paracetamol combination with rosemary alcoholic extract revealed A) little amount of inflammatory cells B) moderate vacuolation H&E X400

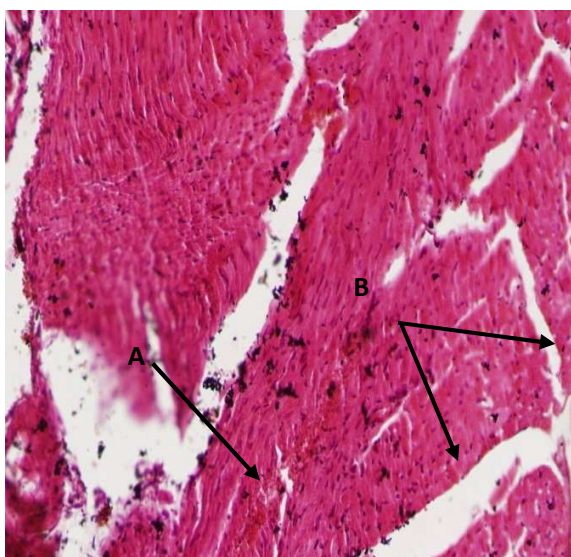


Figure 7 histopathological section of heart muscle in high dose of paracetamol combination with rosemary alcoholic extract reveled A) mild congestion of vessel B)moderate vacuolation H&E X1000

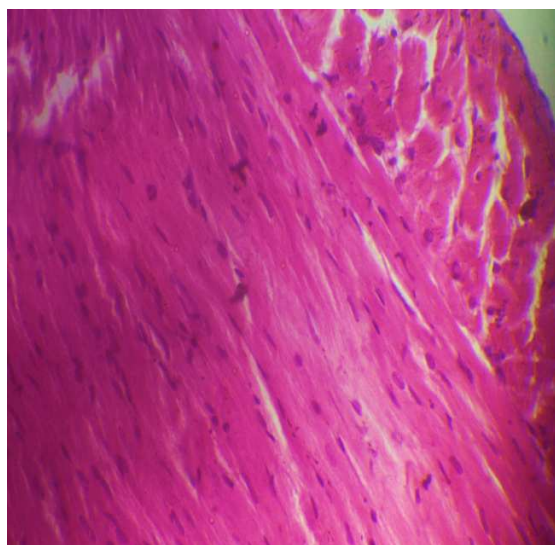


Figure 8 histopathological section of heart muscle when administer rosemary extract after high dose of paracetamol reveled complete treated the heart H&E X1000

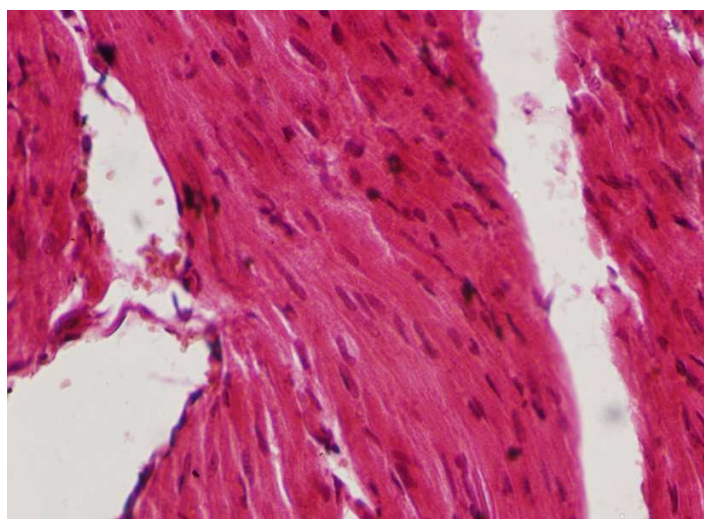


Figure 9 histopathological section of heart muscle when administer rosemary extract after high dose of paracetamol reveled intact cardiac muscle with moderate congestion H&E X400

Discussion

Paracetamol is the most society and often used as antipyretic and analgesic drugs on the universe, that found over the counter (OTC) no need to prescription [17]. High dose of paracetamol for long term leading to a breakdown of the heart muscle either due to the erection of iron or oxidative stress induction due to free radicals. [18] The finding in this study after administration the alcoholic extract of rosemary that showed there is productive and treated effect against worse signs for tacking high dose of paracetamol. The histopathological finding for the cardiac muscle after high dose of paracetamol showed degeneration and hemorrhage our finding agreement with [19,20], whom revealed that the high dose lead to cardio toxicity, myocardial necrosis, and sub endocardial hemorrhages present particularly in the sub endocardium. The physiological mechanism of paracetamol toxicity is well known that accrue due to direct or indirect toxic effects on the myocardium, the indirect toxicity that resulting ischemia, as a result of the exhaustion of sulfhydryl group. Paracetamol exhaustion the sulfhydryl group, that intervene with nitric-oxide manufacture and intervene with endothelial derived vascular relaxing factor both that lead to coronary ischaemia. [19, 21] When given long term of paracetamol in high dose in accompanied with rosemary ethanol extract in group 3 that lead to decreasung the pernicious effect of paracetamol, the antioxidant treatment provide protection against paracetamol -induced myocardial damage. So that led to decrease the side effect of high dose of paracetamol, these findings agreed with [22] That showed the extract of Rosemary leaves owen a highly antioxidants like the flavonoids, phenols, volatile oil and terpenoids, and [12] who found that the Rosemary and its bioactivities especially caffeic acid derivatives such as rosmarinic acid have a therapeutic activity in treatment and prevent the infectious diseases, hepatotoxicity, atherosclerosis, ischaemic heart disease. The results in group 4 in this experiment showed a treatment effect of rosemary alcoholic extract on the heart of experimental rats, the hemorrhage and vacuolation are disappear, that finding agree with [23], whom found the *Rosmarinus officinalis* extracts for leave have the capability to liberation of free-radicals by suppression kinetic signal oxygen and by pitfall and break off the radicals prior they reach a cellular target. The Substantial evidence has shown that regeneration effect of rosemary alcoholic extract are due to presence the active ingredient in this plant that play important role in anti-oxidant and anti-inflammatory this study corroborates with another studies that showed the rosmarinic acid, rosmanol, a hydroxyl cinnamic acid ester, are the major antioxidant

component found in Rosemary officinalies. These component simultaneously with other isoprenoids (sterol, isoprene, mono and diterpenes, tocopherols and carotenoids) are considered as anti-inflammatory factors [22, 24, and 25].

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