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ORIGINAL ARTICLE

Effect of Rutin on the Histological Structure of the Heart and Some Biochemical Indicators of Oxidative Stress in Male Albino Rats

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ABSTRACT

The current study aimed to know the effect of rutin at a dose of (30 mg/kg of body weight) and (60 mg/kg of body weight) on heart tissues in two groups of normal rats and two groups of rats exposed to oxidative stress with 1% hydrogen peroxide for the duration of the experiment., as well as the effects of rutin on the percentage of some antioxidants such as glutathione GSH, and the percentage of malonaldehyde (MDA) in the serum. (48) male rats were used, they were divided into six groups.: the first group (control) included eight males, and the second group hydrogen peroxide H_2O_2 1%, H_2O_2, and rutin at a concentration of 60 mg/kg, H_2O_2 with a concentration of rutin 30 mg/kg, and with a concentration of rutin at a concentration of 60 mg/kg and with a concentration of 30 mg/kg of rutin. All groups dosed daily for one month, after taking blood samples, sacrificing the animals, and making tissue sections of the heart under study. The results revealed that Rutin increased the GSH glutathione ratio in the groups in which oxidative stress was induced by hydrogen peroxide by 1% compared to the control group treated with hydrogen peroxide only. While rutin reduced MDA in the same groups compared to the control group that was treated with hydrogen peroxide by only 1%. At ($P \leq 0.05$) The histological study of the heart of mice treated with Rutin revealed a high cure rate, as Rutin showed protection of the heart tissue from the toxicity caused by hydrogen peroxide. While the tissue sections of the positive control group that were treated with 1% hydrogen peroxide showed clear tissue degeneration with hemorrhage and necrosis.

The histological results of two groups treated with concentrations of (30 mg/kg of body weight) and (60 mg/kg of body weight) showed no toxic effects of rutin compared with the negative control group.

Keywords: Rutin, heart, GSH, MDA

INTRODUCTION

Medicinal plants are considered to have a pivotal role in treating human diseases, as many active substances and medicines are extracted from natural [1]. Bioactive compounds are derived in the form of secondary metabolites that have pharmacological or toxic effects on humans and animals [2], these products are alkaloids, phenolics, and terpenoids [3]. and oxidation [4], due to these properties of flavonoids, has recently attracted the attention of researchers in the medical field [5].

Rutin or RTN, also known as vitamin P, is a nutritional compound from the group of flavonoids consisting of quercetin bound with the disaccharide Rutinose. Because of its anti-cancer and anti-aging properties [6], [7], it also has anti-platelet aggregation, anti-viral and anti-hypertensive properties, as well as supporting and strengthening capillaries [8], rutin is found in a wide range of plants from citrus fruits such as oranges and lemons, and it is found in tomatoes as well as in buckwheat, berries, apricots, and cherries [9] .

Oxidative stress refers to the pathological state of reactive oxygen species (ROS) accumulation resulting from excessive production of oxygen radicals or impairment of the intracellular antioxidant defense system [10]. Oxidative stress also has an important role in regulating the functioning of the heart and blood vessels and has become an important target for the prevention and treatment of cardiovascular diseases. Oxidative stress can cause severe functional damage to endothelial cells and cardiomyocytes [11], [12]. In addition, oxidative stress contributes to the pathogenesis of hypertension, ischemic injury to the myocardium, dysperfusion, atherosclerosis, and other associated diseases by regulating inflammation and stimulating vascular smooth muscle hypertrophy [13], [14].

Data from several epidemiological and clinical studies have shown a positive correlation between the development of CVD and reduced consumption of diets rich in fruits and vegetables [15], [16]. Numerous scientific studies indicate that flavonoid-rich fruits, vegetables, and nuts have many cardiovascular health benefits through antioxidant, free-radical, oxidative stress-causing, anti-inflammatory, and anticoagulant activities through multiple complex mechanisms [15]–[18]. It shows the ability to interact with cell membranes, thus leading to changes in its structure and physical and chemical properties [19] that can alter cell function, protect mitochondria, interfere with and modify enzymatic activities and transcription factors, as well as affect cell gene expression [20], [21].

Several studies have indicated that rutin has a positive effect on reducing the risk of cardiovascular diseases (CVD), it has an important preventive effect in the treatment and prevention of post-thrombotic syndrome [22], and it also reduces the risk of atherosclerosis.

By reducing the cytotoxicity of oxidized LDL cholesterol [23], rutin can prevent the formation of lipid peroxidation, which is one of the most important factors causing the development of various cardiovascular diseases such as myocardial infarction and ischemia [24]. Therefore, the protective properties of flavonoids and rutin are specifically related to the cardiovascular system mainly through their effective anti-oxidative activity [25], [26], either directly by scavenging free radicals or indirectly as inducers of anti-enzymes. for oxidation [27].

MATERIALS AND METHODS

Animals in the study: The experiment was conducted in the animal house of the college of science /University of Al-Kufa,48 male rats that were purchased from the animal house of the college the ages ranged between (16-18) weeks, and their weight ranged between (220-280mg) the experimental animal was placed in a plastic cage, which was 50*35*15 cm in size, with a metal cap,4 rats in each cage in a room of 3*4 meters. All animals were exposed to the same conditions, from a temperature range of 20-25c, organized by an air conditioner and the lighting hours were 13 hours of light, against 11 hours of darkness.

Experimental Design: The animals were divided into six groups at random, with eight animals each for each of my agencies: A first group is a control group that includes 8 animals that were treated with normal saline) during the 30-day trial period. A second group is a treatment group that includes 8 animals that were dosed with the H_2O_2 1% for the duration of the 30-day trial. The third group: Hydrogen peroxide 1% and Rutin compound, 60 mg/kg of body weight, were orally dosed Fourth group: Hydrogen peroxide at a concentration of 1% and Rutin at a concentration of 30 mg/kg of body weight were orally dosed. The fifth group G5: Orally dosed with Rutin compound, a concentration of 60 mg/kg of body weight. The sixth group G6: Orally dosed with Rutin, a concentration of 30 mg/kg. After the 30-day trial period and 24 hours after the last day, the animals were anesthetized with ketamine and xylazine

dissected, and blood samples were obtained from the abdominal vein in non-heparinized tubes to perform the serological tests (GSH and MDA). The autopsy process began with the extraction of the heart.

Microscopy and imaging: After making and coloring the tissue sections, they were cut with a microtome with a thickness of 5 microns. The slides were examined under a compound microscope to determine the tissue changes that resulted in heart tissue and imaged under a microscope outfitted with a digital camera at magnifications of 100x and 400x.

Statistical Analysis: ANOVA analysis and the LSD test were used by (SPSS version 18) to determine the mean for all treatments at the (P≤ 0.05) level (SPSS 2011).

RESULTS

Changes in the level of MDA: The results of the statistical analysis in the table () showed a significant increase ($p < 0.05$) in the level of MDA in the positive control group (G2) compared to the negative control group (G1) and the two groups (G3) treated with rutin at a concentration of 60 mg/kg with H2O2 and (G4) Treatment with Rutin concentration of 30 mg/kg with H_2O_2.

There are also no significant differences between the groups (G3) and (G4) at the level of significance ($p < 0.05$). But when comparing the results of the statistical analysis of the two groups (G5) treated with rutin at a concentration of 60 mg / kg of body weight and (G6) treated with rotten at a concentration of 30 mg / kg of body weight with the negative control group (G1), we notice a significant decrease in the level of MDA. While there is no significant difference in the level of MDA between the groups (G5) and (G6) at the level of significance ($p < 0.05$).

Changes in the level of glutathione GSH: The results of the statistical analysis in table () showed a significant decrease ($p>0.05$) in the level of glutathione GSH in the positive control group treated with 1% hydrogen peroxide (G2) compared to the negative control group (G1), (G3) and (G4).

While the group (G4) recorded an increase in the level of GSH compared to group (G3) at the level of significance ($p < 0.05$). As for the comparison between group G5) and the negative control group (G1), it recorded (G5) a significant increase at the level of significance ($p < 0.05$), and the group (G6) recorded a significant increase compared to the negative control group. While there were no significant differences between the groups (G5) and (G6) at the level of significance ($p < 0.05$).

Table 1: The effect of rutin on parameters of oxidative stress:

Group	GSH (micromole/L)	MDA (micromole/L)
Control	8.32±0.09B	4.58±0.57B
H_2O_2	0.73±0.15E	9.04±1.28A
H_2O_2 + Rutin (60mg/kg)	6.1±0.1D	5.26±0.2B
H_2O_2 + Rutin (30mg/kg)	6.73±0.49C	4.69±0.96B
Rutin (60mg/kg)	9.07±0.1A	3.2±0.1C
Rutin (30mg/kg)	9.66±0.37A	3.17±0.14C
LSD	0.57	1.25

- Value represents the mean ± the standard error.
- Different letters in one column indicate significant differences (P <0.05) between the totals

And the results show that histological sections were taken from the hearts of rats in the first group (the control group) in which the heart tissue appeared naturally, Where the cells appeared spindle-shaped with a central nucleus, and the muscle fibers do not contain spaces between them., as shown in the figure (1). As for the group that was treated with 1% hydrogen peroxide, severe necrosis of the muscle fibers is observed, where most of the muscle fibers appear devoid of the nucleus with a clear degeneration with the presence of spaces between the muscle fibers. ., as shown in figures (2,3). While a slight degeneration is observed, as spaces appear between the cardiac muscle fibers in the group treated with 1% hydrogen peroxide and Rutin at a concentration of 30 mg/kg of body weight.., as shown in the figure (4,5)

A regular and natural arrangement of cardiac fibers is also observed, as it contains a spindle-shaped nucleus that is circumferential to the site. It is also noted that there are narrow spaces between the fibers in the group treated with 1% hydrogen peroxide and Rutin at a concentration of 60 mg/kg of body weight., as shown in the figure (6,7). As for the group treated with Rutin at a concentration of 30 mg/kg of body weight, tissue was observed as normal as in the negative control group, as shown in figure (8). As well as in the group treated with Rutin compound at a concentration of 60 mg/kg of body weight, the tissue was observed almost naturally, as in the negative control group, with the presence of some simple spaces between the fibers., as shown in the figure (9)

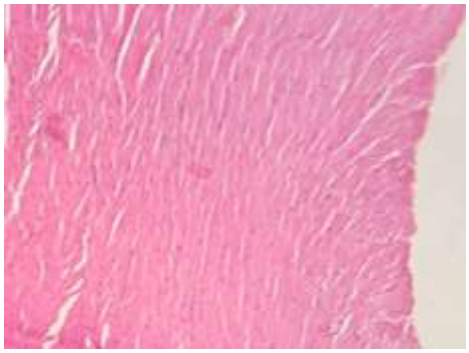

Fig 1: Histological section in the heart of a rat from a G1 40X (H&E).

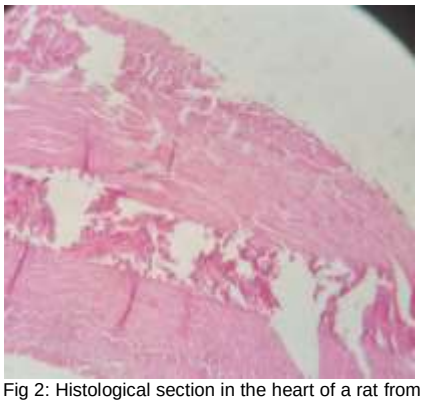

Fig 2: Histological section in the heart of a rat from G2,show severe necrosis of the muscle fibers, where most of the muscle fibers appear devoid of the nucleus, with clear degeneration, with spaces between the muscle fibers. 40 X (H&E)

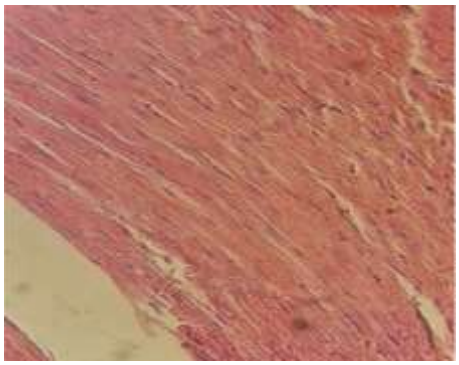

Fig 3: Histological section in the heart of a rat from G3 show that the cardiac fibers have a regular and natural arrangement, which contains a spindle-shaped nucleus that is circumferential to the site. It is also noted that there are narrow spaces between the fibers. 40X (H&E) .

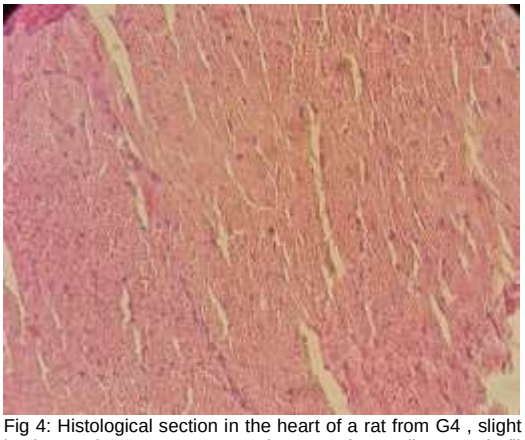

Fig 4: Histological section in the heart of a rat from G4 , slight degeneration is observed, as spaces appear between the cardiac muscle fibers 40X (H & E)

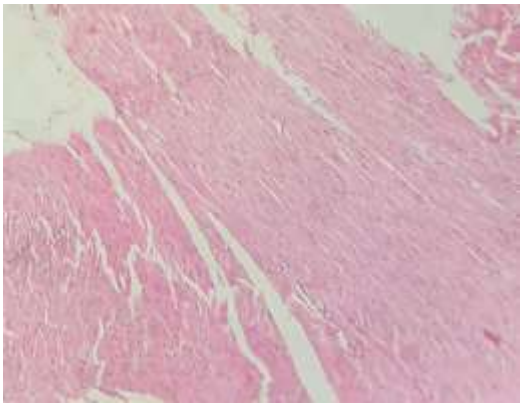

Fig 5: Histological section in the heart of a rat from G5 ,The tissue was observed as normal as in the negative control group X 40 (H&E).

Fig 6: Histological section in the heart of a rat from G6. The tissue is observed almost naturally as in the negative control group, with some minor spaces between the fibers (H&E) X 40.

DISCUSSION

The results showed a significant decrease in the level of GSH in the positive control group (G2) in which oxidative stress was induced by treating it with hydrogen peroxide at 1% for 30 days compared to group (G1). the decrease in the antioxidant enzyme indicates an increase in the production of ROS and a decrease in the antioxidant defense system. [28], The study, with the findings of [29]–[31]. Glutathione is one of the most important first lines of cellular defense against oxidative injury, hence the inefficient detoxification of ROS by antioxidant enzymes may lead to oxidative stress [29], [31].

The results showed a significant increase in the level of GSH in the two groups (G3) treated with rutin at a concentration of 60 mg/kg with H_2O_2 and (G4) treated with rutin at a concentration of 30 mg/kg with H2O2 compared to the positive control group (G2). The antioxidant activity of rutin is attributed to its chemical composition that enables it to scavenge free radicals with high efficiency [32], and rutin helps to increase the production of glutathione and the cellular protection system [33]. Also, rutin has an inhibitory effect on the enzyme Xanthine oxidase involved in the generation of free radicals [34]. The antioxidant activity of rutin is attributed to its chemical composition that enables it to scavenge free radicals with high efficiency [32], and rutin helps to increase the production of glutathione and the cellular protection system [33], and rutin has an inhibitory effect on the enzyme Xanthine oxidase involved in the generation of free radicals [34] These results are in agreement with the researchers' studies [35]–[37].

The results indicated a significant increase in the level of MDA in the positive control group (G2) compared to the negative control group (G1), it is likely that the rise in the level of MDA is due to the cause of increased lipid peroxidation, which usually occurs due to the production of a large number of free radicals and oxidative stress [38], the results of the current study agreed with what was reached [30], [39], [40]. Also, the results showed a significant decrease in the level of MDA in the two groups (G3) treated with rutin at a concentration of 60 mg/kg with H_2O_2 and (G4) treated with rutin at a concentration of 30 mg/kg with H_2O_2, compared to the positive control group (G2). These results are in line with the findings of both researchers [37], [41], where rutin works to break the activation chain of free radicals, inhibits lipid peroxidation activity and free radical production, and reduces the final products of lipid peroxidation [42]. The results also showed a significant decrease in the level of MDA for the (G5) treated with rutin at a concentration of 60 mg / kg and (G6) treated with rutin at a concentration of 30 mg / kg compared with the negative control group (G1). Suppression the effect of lipid oxidation due to their anti-lipid peroxidation and antioxidant and membrane-stabilizing properties. [43].

Histological study: The tissue sections taken from the G2 group (positive control), which was given hydrogen peroxide at a concentration of 1% with drinking water over the length of the experiment (30 days), showed severe necrosis of muscle fibers, where most of the muscle fibers appear devoid of nuclei with clear degeneration with the presence of The spaces between the muscle fibers also notice the presence of a large area of cardiac tissue suffering from degeneration, and this shows the negative effects of oxidative stress and free radicals, which was confirmed by most studies [44].

Studies confirm that free radicals of active oxygen species such as hydroxyl radical, superoxide radical (O_2) and hydrogen peroxide H2O2 lead to the decomposition of cell membranes and nuclei, and then apoptosis [45], [46]. As shown [47] administration of hydrogen peroxide through drinking water leads to the development of oxidative stress and thus a significant increase in the levels of cardiac enzymes in addition to damage to the tissues of endothelial cells of blood vessels and cardiac muscle. The reason for these changes in the tissue of the heart can be due to the fact that hydrogen peroxide has the ability to destroy the plasma membrane and release many chemicals that work to attract inflammatory cells in the area of the injury, thus forming a chain of free radicals that cause tissue damage [48] .

As for the tissue sections taken from the heart of the G3 and G4 groups that were treated with 1% hydrogen peroxide and rutin (60 mg/kg and 30 mg/kg), respectively, there was a clear improvement in the tissues, as not all the pathological effects that appeared in the positive control were seen. It can serve as evidence of the recovery state by the action of the rutin compound after the exposure of muscle tissue to oxidative stress with hydrogen peroxide, as rutin possesses several effective

mechanisms of antioxidant and free radicals mentioned above [49] where it was found that there is a relationship between the ability of flavonoids to inhibit lipid peroxidation and its mechanism of action depending on the chemical composition of flavonoids [50], and this also explains the power of rutin as an antioxidant due to the presence of a hydroxyl group at the C-5, C-7 site in the A ring, and C-3, C-4 in the B ring [51]. Flavonoid such as rutin and quercetin have the ability to activate defense mechanisms in cardiac cells because they are highly effective in scavenging free radicals ROS and reducing oxidative stress in addition to the role that rutin plays in preventing the biosynthesis of enzyme proteins. Which contributes to the redox reactions, where it activates gene expression to form Actine proteins in cardiac muscle fibers to replace the damaged proteins [52].

Histological results agreed with what was stated by the researcher [53] in a study of the effect of rutin on cell death induced by hydrogen peroxide (H_2O_2) in vitro and in vivo in the H_9C_2 cell line derived from the heart tissue of rats with myocardial ischemia. It was reported that rutin provides protection for cardiac tissue from the effects of oxidative stress caused by hydrogen peroxide. As for the fifth and sixth groups G6 and G5, each of which was treated with protein only at a concentration of 60 mg / kg and 30 mg / kg of body weight, respectively, they witnessed, in addition to the normal levels of enzymes CK-MB, LDH, Troponin, Myoglobin, and ions K^+ and Na^+, to the absence of Pathological and negative changes in the tissue sections taken from the animals of these two groups, where the tissue sections appeared similar to the negative control, and this reflects the positive role of rutin as any antioxidant that shows its ability by removing oxygen free radicals and preventing the process of lipid peroxidation of the cell membrane through enzyme inhibition [34]. Xanthin oxidase, and it is proved that the compound of rutin and the two concentrations used in the current experiment (60 mg, 30 mg) does not have side effects or toxicity towards cardiac muscle tissue [54] and many scientific reports agreed that flavonoids such as quercetin Rutin protects cells from tissue-damaging programmed cell death caused by oxidative stress through multiple mechanisms such as inhibition of mitochondrial dysfunction, activation of anti-oxidant enzymes [36].

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