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## The protective effect of rutin supplement in Azithromycin-induced thyroid gland toxicity in male albino rats

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**Abstract:** To evaluate the protective effect of flavonoid Rutin against Azithromycin, in male rats, the present study has been carried out at Al-Qadisiyah University / Faculty of Education / Biology Department, from November to February 2023. The purpose is to study the effect of rutin to reducing the potential effects of azithromycin on the functional and histological thyroid gland in male albino rats.

Forty male albino rats were divided into four groups: the first group (G1) control, was given distiller water orally. The second group (G2), was treated with a dose of (30) mg/kg of azithromycin for ten days, the third group (G3) was treated with a dose of (50) mg/kg of rutin for ten days, and the fourth (G4) was treated by a dose of (30) mg/kg of azithromycin and (50) mg/kg of rutin for ten days.

Results indicated that this caused a significant decline ( $P < 0.05$ ) in T3, T4 and Thyroid Stimulating Hormone (TSH) levels in the plasma of G2 compared with G1. The results also showed a significant increase in (MDA) and a decrease in the level of (SOD) in a group2 (G2) Compared with the control group, while the level of T3, T4, TSH, MDA and SOD was normal in (G3) and (G4), this results proved that used rutin to lessen damage effects of by reduction of generating ROS and ameliorate thyroid toxicity in rats, by restoring normal hormones levels.

According to the histological study's findings, the second group experienced histopathological changes that included smaller follicles with irregular shapes, smaller thyroid follicles with irregular lining layer thickness, the development of hyperplasia, and finally and bubbles in the colloidal substance.

**Keywords:** rutin, azithromycin, reduction, damage, Thyroid gland

## 1. Introduction

Some plants and herbal extracts have been used since ancient times to relieve symptoms of disease [1] Chemical

compounds derived from medicinal plants are also safer than similar synthetic medicines [2]. Flavonoids are widely found in plants and the human diet, and they are secondary metabolites of multiple phenolic compounds. Flavonoids

are included in the composition of many drugs and have become a subject of medical research because they have many biological, medical and pharmaceutical properties, as they act as antioxidants, antivirals, and anti-inflammatory. Anti-mutagenic, anti-tumor and anti-cancer, and work to protect the heart and blood vessels. [3,4]

Rutin is a non-toxic bioflavonoid. Rutin consists of the flavonol quercetin and the disaccharide rutinose. Vitamin P is another name for rutin, Szent Gyorgi a scientist, discovered it while conducting significant research on anti-cancer flavonoids. [5]. Also called sophorin, rutoside, or quercetin-3-retinoid, it derives its name from the name of the plant 'Ruta grave lens, which contains it in large quantities [6]. Rutin is also used as an anti-inflammatory agent due to its ability to remove free radicals [7], and thus it can be effective in treating chronic inflammatory diseases [8].

It has anti-inflammatory activity as it reduces the number of basophils and eosinophils in pulmonary secretions, which suppresses asthma symptoms [9]. Also, rutin has a protective effect on lung cells and tissues in acute pulmonary infections, as Showed a protective effect on histopathological abnormalities in infected lung tissues and reduced lipid peroxide formation [10]. Rutin also has anti-allergic activity by inhibiting basophils to secrete histamine [11], and it also works to a large extent to prevent the formation of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), which is one of the main factors inducing inflammation. Liver injury with carbon tetrachloride (CCl<sub>4</sub>) has a protective role for rutin by increasing the levels of antioxidant enzymes and decreasing the levels of lipid peroxidation in the liver [12]. Also, rutin has a protective effect against cirrhosis resulting from oxidative stress, which is one of the causes. underlying liver cirrhosis, and therefore the use of antioxidants can be a unique method of treatment [13].

Azithromycin is a broad-spectrum antibiotic derived from the antibiotic erythromycin,

which is a class of macrolides that includes many well-known antibiotics, including clarithromycin, erythromycin, and fidaxomycin [14,15]. Azithromycin is widely used in many medical conditions. In addition to its antibacterial activity, its effective roles against various pathogens have been revealed. Macrolides are among the safest antibiotics available, despite their association with some side effects and events that resulted from their use [16]. Headache and dizziness are among the most common side effects for patients receiving macrolide treatment, with a rate of up to 1.5% of patients. The thyroid gland is a part of the body's endocrine system. It is the largest specialized organ of endocrine glands in the human body. The thyroid gland is necessary for the normal growth of the body, It is composed of follicles lining with epithelial cells, filled with a colloidal substance made up of Thyroglobulin protein which is binding with iodine, this substance is the only source of the two types of thyroid hormones T<sub>3</sub>, T<sub>4</sub>[17].

The thyroid gland is the main responsible for the metabolic process and plays an essential role in regulating the basic metabolic rate in the body of the organism and stimulating psychological growth in addition it has a vital role in calcium metabolism, it is distinguished from the other glands by its ability to absorb iodine and produce hormones and store it is inside the gland itself, and secretes hormones when needed them. The thyroid gland is one of the endocrine glands because secretes its hormones directly into the bloodstream [18]. The biosynthesis and secretion of thyroid hormones (T<sub>3</sub>, T<sub>4</sub>) are stimulated by thyroid stimulation hormone (TSH), and controlled by (negative feedback mechanism) for the axis of the hypothalamus - pituitary gland - thyroid gland.

## 2. Methodology

### Experimental animals

This study, which employed 40 male albino rats obtained from the animal's house of the Faculty of Science at Al-Kufa University and

conducted in the Biology Department of the Faculty of Education's Al-Qadisiyah University, their average weights were between (110-120) grams. The animals were placed in plastic cages 15 x 35 x 50 cm dimensions, with 10 animals per cage, in a room of 3 x 4 meters. All animals were subjected to the same 12 hours of light and 12 hours of darkness, with an air conditioner-controlled temperature of 20 to 22 °C. Animals received focused free food and water. Ten animals each made up each of the four groupings that were created.

**Animal sacrifice:** After 24 hours from the last dose, the experimental animals were anaesthetized by using (xylaine and ketamin) Partially and then withdrawn blood by medical syringes of 5 cc directly from the heart while the rats are anaesthetized by Heart Puncture. The blood samples were placed in gal tubes, then we used a centrifuge for fifteen minutes at a speed (3000 r/min) to obtain the blood serum and micropipette. Until the tests were conducted, the serum was maintained in labelled Eppendorf tubes and kept it at a temperature of (-20) °C. After the thyroid gland was taken from the animals, they were dissected to prepare tissue sections. Rutin was purchased from Sigma-Aldrich chemical company (St Louis, MO, US).

Azithromycin from the local pharmacy.

#### **Animals' groups:**

- 1- Control group (G1): is given distinctly for 10 days.
- 2- Second group (G2) : Azithromycin is given at a dosage 30 mg/ kg for 10 days.
- 3- Third group (G3): Rutin is given at a dosage 50 mg/gk for 10 days.
- 4- Forth group (G4): Both azithromycin and rutin are given at the same doses and period.

#### **Studying some biochemical parameters**

##### **- Determination of T3 and T4 concentration.**

Using ELISA technology, a measurement of T3, T4 was made using a pre-made test kit. Using a kit (KIT) made by (Sunlong Biotech

Chinese), read the absorbance O.D. at 450 nm with a Microtiter Well Reader.

##### **- Determination of (TSH) concentration**

The assay depends on the reaction between the antibodies in the reagent enzyme and the hormone antigens (TSH) in the serum in the KIT to determine the TSH hormone, and we use the ready-made test kit. The manufacturer of the company is Chinese Sunlong Biotech. The absorbance was then read using a Microtiter Well Reader at 450 nm.

##### **- Determination of superoxide dismutase (SOD)**

The researcher's [19] approach was used to determine the concentration of (SOD) in rats' serum samples. The projected absorption value is 560 nm.

##### **- Determination of Malondialdehyde (MDA)**

The method of researchers [19] was used to measure the concentration of (MDA) in rats serum samples. The anticipated absorbance value is 532 nm.

#### **Histological examinations**

According to the researchers' method [20] the tissue sections were prepared. The samples were washed with tap water for an hour and the fixative was removed. The thyroid follicles diameter and the thickness of the epithelium lining them were measured by using the scale Ocular micrometer power of 10 X, sections were examined and then the general average was calculated to extract the average diameter of the thyroid vesicles and the thickness of their lining layer for each group.

#### **Statistical analysis**

Using the test (F), the statistical analysis findings were examined to determine whether there were any significant differences between the treatment groups and the control group. These differences were found to be significant at a threshold of 5% probability. [21]

### 3. Results And Discussion

#### Measurement of the concentrations of thyroid hormones T3 and T4

The results of the statistical analysis shown in Table (4-1) showed a significant decrease ( $P<0.05$ ) in the concentrations of T3 and T4 hormones in the serum of experimental animals in the second group (G2), which were treated with azithromycin for (10) days. As for the third group, G3, which was treated with rutin for 10 days, respectively, a significant increase was observed in the concentration of T3 and T4 compared to the control group, as well as compared to the groups G2. While the results of the statistical analysis showed that there were no significant differences ( $p>0.05$ ) between the fourth group G4 which was treated with the drug and rutin together for 10 days, respectively, when compared with the control group G1, but we found a significant difference ( $P<0.05$ ) when compare the results with the second groupings G2.

The results of this study showed that there was a significant decrease in the thyroid enzymes T3 and T4 in the groups that were treated with azithromycin, and this decrease occurred due to the toxic effects caused by the drug on the thyroid gland cells through its generation of free radicals and ROS, and this is consistent with the results presented by the researchers [22,23] who indicated that azithromycin works to generate free radicals that were the main cause of hepatotoxicity and significant damage to cell membranes, which caused leakage of hepatic enzymes into the serum. Researcher [24] his group (2017) indicated that macrolides induce hepatotoxicity through the production of free radicals and the production of ROS. On the other hand, a study conducted by [25] his group (2018) indicated an increase in the levels of urea and creatinine, which are metabolites filtered by the renal glomeruli, and they are two main indicators of the function of the kidneys. He attributed this rise to the oxidative stress caused by the drug, as free radicals can interact with Oxygen to produce reactive oxygen species (ROS) that

stimulate lipid peroxidation in glomerular epithelial cells, which destroys the structure and function of the filtration membrane.

The significant improvement in the levels of T3 and T4 hormones in groups G3, G4, which were treated with rutin alone or with rutin and the drug simultaneously when compared with the control group, can be attributed to the positive effect of rutin as an antioxidant and reduce the negative effects of azithromycin. And this is by reducing the damage of oxidative stress resulting from the use of the drug, researcher [26] his group (2015) mentioned that rutin played an important role in reducing the negative effects of azathioprine (a chemotherapy treatment) on the tissues of the pituitary gland and testis from the damage of oxidative stress that cells are exposed to as a result of accumulation Free radicals generated by the treatment by increasing the effectiveness of antioxidants. Rutin also works to increase adipose tissue, the hypothalamus and the pituitary gland, as well as improve the process of iodine uptake by thyroid cells, which in turn was reflected in the increase in the secretion of thyroid hormones [27].

#### Concentration measurement of TSH

The results of the statistical analysis of this study showed that there was a significant decrease ( $p<0.05$ ) in the concentration of TSH in the two groups G2, which were treated with azithromycin at a concentration of 30 mg/kg of body weight for 10 days, respectively, when comparing the results with the control group G1, while There were no significant changes ( $p>0.05$ ) in the two groups G3, which were treated with rutin at a concentration of 50 mg/kg for 10 days, respectively, as well as the group G4, which were treated with the drug and rutin together at the same concentrations and periods above when compared with the control group as in schedule.

The reason for this decrease may be the consumption of antioxidants and the high oxidative stress caused by the drug, and this result is consistent with what was reached by

the researcher [28] his group (2002), who proved that the toxic effects of azithromycin on liver cells arise and develop through the production of ROS and reduce the levels of antioxidants. The reason for the low level of TSH may be due to an increase in DNA breakdown as a result of oxidative stress resulting from high levels of ROS, which interacts with cellular molecules such as DNA and mRNA. Cell necrosis and the cause of DNA breakdown have been explained by increased levels of ROS and RNS that interact with DNA and cause irreversible condensation of chromatin in the cell nucleus. The reason for the decrease in the level of TSH, T3, and T4 hormones may be the decrease in the level of the TRH hormone, which is secreted from the hypothalamus as a result of the decrease in the mRNA level of the TRH hormone due to the negative effects of the drug.

As for the groups that were treated with rutin alone or treated with rutin and the drug simultaneously, the results of the study showed that there were no significant differences when compared with the control group, and this is due to the ability of flavonoids, including rutin, in cells and tissues. The tissues of the reproductive organs, the harmful effects of free radicals generated by the drug azathioprine, also, rutin contributed to increasing the levels of hormones (LH, FSH) secreted by the pituitary gland Hypothalamic-pituitary axis. On the other hand, the researcher [29] his group (2019) mentioned that rutin has shown a moderate ameliorate effect on the toxicity of carbon tetrachloride (CCL<sub>4</sub>) on the tissues of the liver, kidneys, and gonads in male white rats, as the researcher found that rutin can scavenge free radicals and inhibit stress. oxidative damage and supports the enzymatic antioxidant system in tissues and thus works to protect cells and tissues.

The results of the current study showed a significant increase ( $P < 0.05$ ) in the level of MDA, and a significant decrease ( $P < 0.05$ ) in the level of SOD in group G2, which was treated with azithromycin at a concentration of

30 mg/kg, compared with the control group. While the levels of MDA and SOD were normal in group G3, which was treated with rutin when compared with the control group. Also, the results of this study did not show significant differences ( $P > 0.05$ ) in the levels of MDA and SOD in group G4 when compared with the control group.

The results of this study agree with a study conducted by the researcher [30] and his group (2015), as they indicated that the drug azithromycin causes an increase in the levels of MDA with a decrease in the levels of CAT, GSH, and SOD and that the reason for this may be due to an imbalance between antioxidants and free radicals, in other words. Disturbances in oxidative stress cause structural and functional damage in cells, and it is known that macrolides alter the redox balance, which leads to oxidative stress and lipid peroxidation [31] As for the groups that were dosed with rutin alone or dosed with rutin and the drug simultaneously, MDA levels were almost normal. The reason for this is due to the ability of rutin to inhibit the ability of metal ions that are capable of superoxide- Fat hyper oxidation and reduction of its final products and also works to break the activation chain of free radicals [32].

Table (1) shows the effect of Azithromycin, Rutin and Azithromycin &Rutin on some biochemical parameters in male rats.

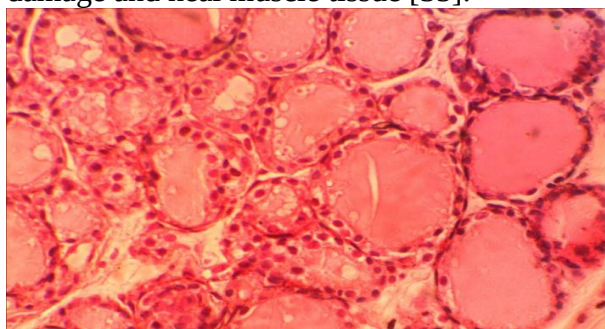
| Group s | TSH<br>Nanog/<br>ml | T4<br>Nanog/ml   | T3<br>Nanog/m<br>l | MDA<br>Nanog<br>/ml  | SOD<br>Nanog<br>/ml |
|---------|---------------------|------------------|--------------------|----------------------|---------------------|
| G1      | 1.7±0.1<br>6A       | 37.96±1.2<br>9A  | 1.6±0.42<br>A      | 13.67±<br>0.97A      | 0.818±<br>0.03A     |
| G2      | 0.89±0.<br>94C      | 14.96±6.9<br>3C  | 1.04±0.0<br>7B     | 28.84±<br>11.8B      | 0.508±<br>0.01B     |
| G3      | 1.43±0.<br>12ABC    | 37.23±±2.<br>22A | 1.42±0.4<br>1AB    | 13.22±<br>8.31A      | 0.809±<br>0.21A     |
| G4      | 1.41±0.<br>18ABC    | 32.61±4.9<br>5A  | 1.21±0.2<br>1AB    | 16.85±<br>11.99<br>A | 0.791±<br>0.13A     |
| LSD     | 0.54                | 11.85            | 0.509              | 12.53                | 0.24                |

#### Histopathological study:

Pathological changes were seen in the second group (G2) as in Figure (1,2,3,4), when

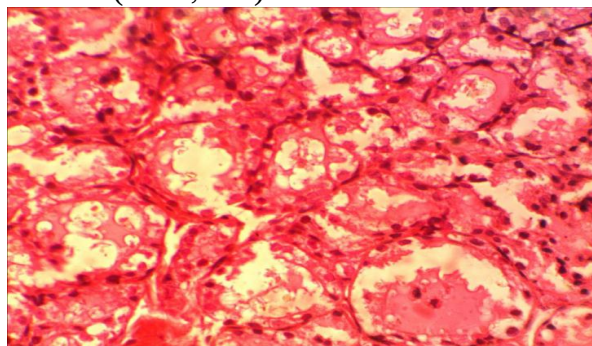


the thyroid gland's histological slides were examined. These changes included the presence of numerous small irregular thyroid follicles, colloidal substance bubbles, the thickness of the vesicle lining layer was increased, some empty vesicles, hyperplasia, and more than one layer of epithelial cells lined some vesicles. The reason for these changes may be due to azithromycin's ability to destroy DNA due to an increase in oxidative stress brought on by the increase in levels of reactive oxygen species (ROS), as demonstrated by the current study. Azithromycin also causes rupture of the hepatic cords, changes in adipose tissue, congestion and severe bleeding in the sinusoids, changes in adipose tissue, and vacuolar degeneration. [ 33,34] The histological sections of the G3 group, in contrast, showed normal thyroid follicles of large, medium-sized, and small size that were lined with epithelial cells and filled with colloid. This indicates that, at this dose, the rutin compound has no toxic or adverse effects on the thyroid gland. According to researcher [34] . According to a study by [35], rutin has no unfavourable side effects and does not affect the heart muscle tissue. Rutin had been demonstrated that it can mend muscle tissue that has been exposed to oxidative stress from hydrogen peroxide, and the histological sections of the fourth group (G4) looked similar to sections of the control group. Rutin has many effective antioxidant and free radical mechanisms previously mentioned, and nutrition rich with flavonoids works to reduce damage and heal muscle tissue [35].

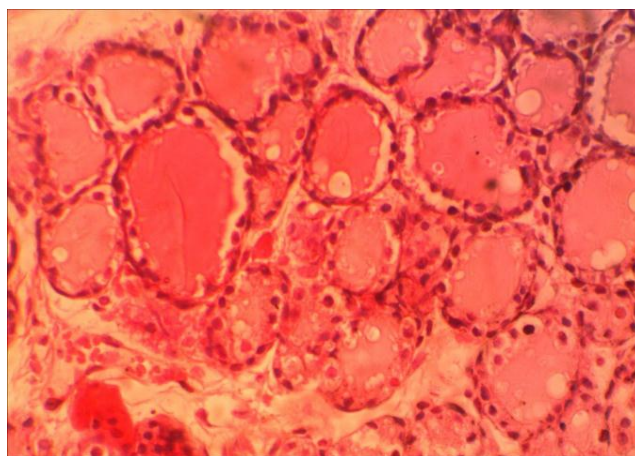


Figure(1) control group showed normal thyroid follicles in big, middle and small sizes

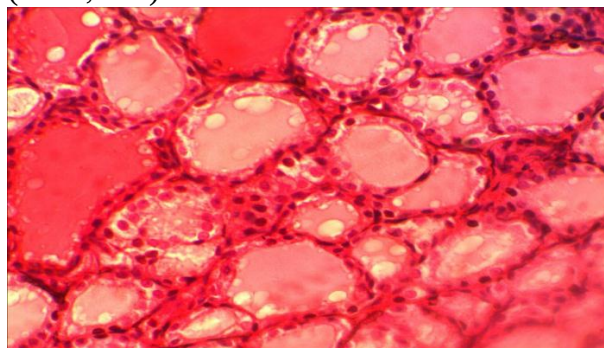
lined with epithelial cells and filled with colloid. (H&E,40X)



Figure(2)Azithromycin group bubbles in the colloidal solution, some empty vesicles, small, irregular thyroid follicles, hyperplasia, and an increase in the thickness of the lining layer (H&E,40X)



Figure(3) Rutin group showed normal thyroid follicles in big, middle and small sizes lined with epithelial cells and filled with colloid. (H&E,40X)



Figure(4) Azithromycin &Rutin group showed normal thyroid follicles big, middle and small size lined with epithelial cells and filled with colloid. (H&E,40X)

## Conclusion

The results showed that the histological studies of (G2) included smaller thyroid follicles with irregular shapes, smaller thyroid follicles with increased lining layer thickness, the development of hyperplasia, and finally colloids and bubbles in the colloidal substance.

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