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COMPARISON STUDY BETWEEN MARJORAM (ORIGANUM MAJORANA L.) AND THYME (THYMUS VULGARIS L.) OILS AGAINST GENTAMYCIN-INDUCED NEPHROTOXICITY IN RATS

Marwa A. Ahmed[1*]

[1*]Department of Food Science and Nutrition, College of Science, University of Bisha, P.O. Box 551, 61922, Bisha. Saudi Arabia, Email: marwa@ub.edu.sa, ORCID ID: 0009-0009-5885-3528

ABSTRACT

Background: Gentamicin-induced kidney failure is primarily caused by oxidative damage, inflammation, and apoptosis. Therefore, marjoram contains phenolic terpenoids including (thymol and carvacrol), flavonoids, tannins, hydroquinone, phenolic glycosides, moreover, thyme oils contain thymol and carvacrol as their main bioactive components that all considered preventive agents against gentamycin-induced nephrotoxicity in rats. Objective: To compare the effects of Marjoram (Origanum majorana L.) and Thyme (Thymus vulgaris L.) Oils Against gentamycin-induced nephrotoxicity in rats. Materials and Methods: Fifty five male albino rats weighing 200±5g were divided into equal five groups (n=5 rats); one kept as normal control negative (C -ve), while the rest four groups were via intra-peritoneal injected by gentamicin at a dose of 100 mg/kg b. wt., for 7 days to induce nephrotoxicity; one group left as a positive control (C +ve), while the rest three groups fed on 1 mL/kg b. wt., of marjoram, thyme oils and mixture of them each, for 45 consecutive days. At the end of experiment, body weight gain, food intake, feed efficiency ratio and relative organs weights were determined, Moreover, kidney functions (creatinine, urea and uric acid), total protein, albumin and globulin were determined, as soon as histopathological examinations of kidneys had been measured. Data were analyzed using the Statistical Package for Social Sciences (SPSS) ver. 28. Results: Data showed a significant decrease in body weights gain and relative organs weights. Moreover, there was a significant decrease in creatinine, urea, uric acid, total protein, albumin and globulin as soon as noticeable kidneys histopathological improvement. Conclusion: It was proven that marjoram and thyme oils could be used as treatment agents for gentamycin-induced nephrotoxicity in rats indicating more effectiveness for thyme oil.

Keywords: Nephrotoxicity, marjoram oil, thyme oil, rats, histopathology.

INTRODUCTION

Gentamicin (GM) is the oldest aminoglycoside antibiotic, commonly used to treat renal illness and intense infections caused by Gram-negative bacteria. Nevertheless, GM produces significant ototoxicity and is harmful to the renal system, which restricts its continued administration 1. In addition, GM exhibits dose-related nephrotoxicity and accounts for 20% of all cases of acute kidney failure 2. The types of kidney damage associated with GM therapy include renal tubular necrosis, tubular edema, glomerular damage, renal apoptosis, and changes in kidney function markers. GM induces kidney damage by selectively accumulating in the epithelial cells of proximal convoluted tubules in the renal tissues 3. Reactive oxygen species are produced and renal antioxidant enzyme activities are inhibited when GM accumulates in renal tubular cells4. GM nephrotoxicity is related to the occurrence of renal oxidative stress, which plays an important role in increasing the proinflammatory cytokines that cause renal apoptosis and impair various renal functions 5. In tubular cells, GM triggers different pathological events such as oxidative stress, inflammation, apoptosis, necrosis, phospholipidosis, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, etc. These events can lead to cell death, tubular dysfunction, and a decrease in glomerular filtration rate (GFR) 6 . Origanum majorana L. (O. majorana) is a member of the mint family Lamiaceae. Typically, products identified as marjoram are the dried leaves and flowering tops of O. majorana L, which is found throughout the world. It contains phenolic terpenoids (thymol, carvacrol), flavonoids (diosmetin, luteolin, apigenin), tannins, hydroquinone, phenolic glycosides (arbutin, methyl arbutin, vitexin, orientin, thymonin), triacontan, sitosterol, acids (oleanolic acid) and cis-sabinene hydrate 7 8 . Thyme (Thymus vulgaris L.) has been used in folk medicine for the treatment of asthma and bronchitis. It is used as an antiseptic, antitussive, antifungal, antiviral, antidiabetic, and antioxidative agent 9. TV oils contain thymol and carvacrol as their main bioactive components. Both of them may possess the same pharmacological effects as TV. TV also has many polyphenolic compounds that are responsible for its antioxidant effects such as rosmarinic acid, quercetin, eriocitrin, luteolin, and epigenin 10. Accordingly, this investgation aimed to compare the effects of Marjoram (*Origanum majorana L.*) and Thyme (*Thymus vulgaris L.*) Oils Against gentamycin-induced nephrotoxicity in rats.

MATERIAL AND METHODS

a. Materials

1. Oils preparation: The oils of marjoram and thyme were purchased from Al-Harraz shop, Cairo, Egypt as a purified oil.

2. gentamicin: Obtained as a drug from the Farmacy.

3. Rats: purchased from Cairo Food Technology Research Institute experimental animal station, Cairo, Egypt.

b. Methods

1. Biological investigation:

Fifty five Sprague Dawley male albino rats weighing 200±5g were housed individually in well-aerated cages and fed on basal diet for one week for adaptation. The basal diet consisted of 70% corn starch, 10% casein, 10% corn oil, 4% salt mixtureture, 1% vitamin mixtureture, and 5% cellulose, as recommended by 11.

2. Preparation of renal failure rats:

Intraperitoneal injection of gentamicin at a dose of 100 mg /kg b. wt., for 7 days to induce renal failure for experimental rats according to the method described by 12.

3. Experimental Design:

Fifty five male albino rats weighing 200±5g were divided into equal five groups (n=5 rats); one kept as normal control negative (C -ve), while the rest four groups were via intra-peritoneal injected by gentamicin at a dose of 100 mg/kg b. wt., for 7 days to induce nephrotoxicity; one group left as a positive control (C +ve), while the rest three groups fed on 1 mL/kg b. wt., of marjoram, thyme oils and mixture of them each, for 45 consecutive days. During the experimental period, rats feeding was recorded, moreover, body weight was recorded every week. At the end of the experimental period, rats were fasted overnight before sacrificing. Blood samples were collected from aorta, in dry clean centrifuge tube, and left for 15 minutes to clot at room temperature, then centrifuged for 15 minutes at 3000 r.p.m for serum separation. Kidneys, liver, and heart were removed, cleaned, weighed then saved in 10% formalin solution for histopathological examination.

4. Biological Evaluation:

Body weight gain (BWG), food intake (FI), feed efficiency ratio (FER) were calculated according to 13 . Using the following formulas.

BWG= Final weight – Initial weight

$$FER = \frac{\text{Gain in body weight (g)}}{\text{Food Intake (g)}}$$

5. Analytical methods:

Blood serum creatinine, blood urea nitrogen (BUN) and uric acid levels, as markers of renal function, were determined 14-16 , respectively. Serum total protein, albumin and globulin were determined 17.

6. Histopathological examination:

The specimens for kidneys were fixed in 10% neutral buffered formalin (pH=7.0), dehydrated in ethyl alcohol and cleared in xylol then embedded in paraffin; stained of 4-6 microns thickness sections with haematoxylin and eosin for examining the kidneys using light microscope at various magnification 18.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) ver. 28. Comparing of the 5 groups were conducted by the analysis of variance (ANOVA) using the procedure reported by 19. The treatment means were compared using the least significant difference test (LSD) at a 5% level of probability 20.

RESULTS

1. Effect of marjoram and thyme oils and a mixture of them on BWG, FI and FER for nephritis in rats:

Body weight gain (BWG), food intake (FI) and feed efficiency ratio (FER) of control (-ve), control (+ve) and treated rats with marjoram and thyme oil 1 ml/kg b.wt. after inflicting with renal failure are showed in table (1).

Table (1): Effect of delonix regia on body weight gain (BWG), food intake (FI), and feed efficiency ratio (FER) in rats inflicted with renal failure

Parameters / Animal group	BWG (g/45d)	FI (g/45d)	FER
Control (-)	17.55±0.98 e	29.25±0.28 e	0.60±0.03 d
Control (+)	47.33±2.27 a	89.07±0.91 a	0.53±0.08 e
Marjoram oil 1 ml/kg b.wt.	37.00±1.37 d	41.08±0.77 d	0.90±0.04 a
Thyme oil 1 ml/kg b.wt.	39.33±1.18 c	55.99±0.41 c	0.70±0.03 b
Mixture of oils 1 ml/kg b.wt.	42.17±1.91 b	65.37±0.43 b	0.65±0.05 c

Values denote arithmetic means ± standard deviation of the means.

Means with different letters (a, b, c, d) in the same column differ significantly at $p<0.05$.

Using one way ANOVA test, while those with similar letters are non-significant.

It could be cleared for rats injected with gentamicin (control +ve) group that body weight gain (BWG) was 47.33±2.27 compared with 17.55±0.98 g/45 day in control (-ve) normal rats ($p<0.05$). These results indicate that there was significant increase in BWG of control +ve compared with control -ve group. All rats injected with gentamicin then marjoram oil 1 ml/kg b.wt., thyme oil 1 ml/kg b.wt., and the mixture of them showed significant decrease in BWG compared with control positive group.

As for food intake (FI), there was significant increase in control +ve group which was 89.07±0.91 g/45d compared to 29.25±0.28 g/45d for control -ve group. Moreover, there were significant decrease in gentamicin injected groups then treated with marjoram oil 1 ml/kg b.wt., thyme oil 1 ml/kg b.wt., and the mixture of them as compared to control positive group. As soon as the hiegest significant decrease in FI for marjoram oil 1 ml/kg b.wt., which was 41.08±0.77 g/45d.

Regarding feed efficiency ratio (FER), the control +ve mean was 0.53±0.08 compared to 0.60±0.03 for control. These data show that there were significant decrease in FER in control +ve rats compared with control -ve rats. All rats injected with gentamicin then treated with all treated group showed significant increase in FER compared control positive group showing that the highest significant increase was for marjoram oil 1 ml/kg b.wt.

2. Effect of marjoram and thyme oils and a mixture of them on relative kidneys, liver and heart weights in rats inflicted with renal failure:

Data for relative kidneys, liver and heart weights for normal, nephritis and treated rats with marjoram and thyme oils at a dose of 1 ml/kg b.wt. after inflicting with renal failure are recorded in table (2).

Table (2): Effect of marjoram and thyme oils on relative kidneys, liver and heart weights in rats inflicted with renal failure

Parameters / Animal group	Kidneys (g)	Liver (g)	Heart (g)
Control (-)	1.78±0.02 e	5.25±0.08 e	0.82±0.04 e
Control (+)	2.18±0.05 a	9.22±0.09 a	1.99±0.07 a
Marjoram oil 1 ml/kg b.wt.	1.88±0.03 d	6.02±0.02 d	1.31±0.05 b
Thyme oil 1 ml/kg b.wt.	1.80±0.05 c	6.44±0.04 c	0.97±0.02 cd
Mixture of oils 1 ml/kg b.wt.	2.10±0.04 b	7.91±0.03 b	1.00±0.02 c

Values denote arithmetic means ± standard deviation of the means.
Means with different letters (a, b, c, d) in the same column differ significantly at $p<0.05$.
Using one way ANOVA test, while those with similar letters are non-significant.

It could be observed for gentamicin (control +ve) group that relative kidneys, liver and heart weights were 2.18±0.05, 9.22±0.09 and 1.99±0.07g, compared to 1.78±0.02, 5.25±0.08 and 0.82±0.04g in (C-ve) normal rats ($p<0.05$). These results denote that there were significant decrease relative kidneys, liver and heart weights for (control +ve) group compared with (control -ve) group. All groups injected with gentamicin then treated with marjoram oil 1 ml/kg b.wt., thyme oil 1 ml/kg b.wt., and the mixture of them should significantly increase in relative kidneys, liver and heart weights as compared to control positive group.

3. Effect of marjoram and thyme oils and a mixture of them on kidneys functions for nephritis in rats:

The effect of marjoram and thyme oils and a mixture of them at a dose of 1 ml/kg b.wt. on kidneys functions for nephritis in rats is shown in table (3).

Table (3): Effect of marjoram and thyme oils on creatinine, urea and uric acid in rats inflicted with renal failure

Parameters / Animal group	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Control (-)	0.96±0.09 e	39.87±1.64 e	1.85±0.03 e
Control (+)	1.33±0.09 a	69.58±2.51 a	2.70±0.09 a
Marjoram oil 1 ml/kg b.wt.	1.31±0.07 b	44.13±1.30 bc	2.30±0.06 c
Thyme oil 1 ml/kg b.wt.	1.01±0.03 d	45.45±1.72 d	2.20±0.03 cd
Mixture of oils 1 ml/kg b.wt.	1.12±0.02 c	42.50±0.53 d	2.60±0.02 b

Values denote arithmetic means ± standard deviation of the means.
Means with different letters (a, b, c, d) in the same column differ significantly at $p<0.05$.
Using one way ANOVA test, while those with similar letters are non-significant.

It is cleared from the data that there were significant increase in the mean of serum levels of creatinine, urea and uric acid in control +ve groups compared with control -ve groups which were 1.33±0.09, 69.58±2.51, 2.70±0.09, 0.96±0.09, 39.87±1.64 and 1.85±0.03 mg/dL, respectively. All rats orally injected with gentamicin then treated with marjoram oil 1 ml/kg b.wt., thyme oil 1 ml/kg b.wt., and the mixture of them showed significant decrease in creatinine, urea and uric acid compared to control positive groups. The highest significant decrease in creatinine and urea serum levels were shown in thyme oil 1 ml/kg b.wt., groups compared to control positive groups which were 1.01±0.03, 45.45±1.72, 1.33±0.09 and

69.58±2.51 mg/dL., respectively. Moreover, marjoram oil 1 ml/kg b.wt., thyme oil 1 ml/kg b.wt., individually groups showed the highest significant decrease in uric acid compared to control positive group.

4. Effect of marjoram and thyme oils and a mixture of them on T.P, Alb. and Glob., for nephritis in rats:

Serum total protein (T.P), albumin (Alb.) and globulin (Glob.) in rats inflicted with renal failure then treated with marjoram and thyme oils and a mixture of them at a dose of 1 ml/kg b.wt. are listed as mean values ± standard errors, in table (4).

Table (4): Effect of marjoram and thyme oils on T.P, Alb. and Glob., in rats inflicted with renal failure.

Parameters / Animal group	T.P (mg/dL)	Alb. (U/L)	Glob. (U/L)
Control (-)	7.13±0.71 e	18.27±1.99 e	25.85±0.49 e
Control (+)	15.96±0.27 a	39.90±3.29 a	45.35±1.69 a
Marjoram oil 1 ml/kg b.wt.	11.37±0.86 d	35.40±2.04 b	41.09±0.38 b
Thyme oil 1 ml/kg b.wt.	9.89±0.14 b	33.08±3.28 c	39.25±0.27 bc
Mixture of oils 1 ml/kg b.wt.	8.02±0.38 c	29.15±2.77 d	30.37±0.45 d

Values denote arithmetic means ± standard deviation of the means.
Means with different letters (a, b, c, d) in the same column differ significantly at $p<0.05$.
Using one way ANOVA test, while those with similar letters are non-significant.

It is cleared from the table that there were significant increase in the of serum levels of total protein, albumin and globulin in control positive groups when compared to control negative groups which were 15.96±0.27, 39.90±3.29, 45.35±1.69, 7.13±0.71, 18.27±1.99 and 25.85±0.49 mg/dL, respectively. All treated groups by marjoram oil 1 ml/kg b.wt., thyme oil 1 ml/kg b.wt., and the mixture of them showed significant decrease in all previous parameters compared to control positive. The highest significant decrease was found in Marjoram oil 1 ml/kg b.wt., for total protein, meanwhile, the highest significant decrease was found in Mixture of oils 1 ml/kg b.wt. group for albumin and globulin.

DISCUSSION

The study declared the effects of Marjoram (Origanum majorana L.) and Thyme (Thymus vulgaris L.) Oils Against gentamycin-induced nephrotoxicity in rats. It was cleared that marjoram and thyme oils could be used as treatment agents for gentamycin-induced nephrotoxicity in rats indicating more effectiveness for thyme oil. The treatment containing thyme decreased the concentration of glucose, heterophil, and heterophil to lymphocyte ratio and increased the concentration of glutathione peroxidase, white blood cells, and lymphocytes ($P<0.05$). A significant increase in water holding capacity and a significant decrease in cooking loss, dripping loss, and thiobarbituric acid of breast meat were observed in the treatment containing thyme ($P<0.05$). The positive effects of thyme on reducing the negative effects of high stocking density and improving growth performance, antioxidant status, blood immunity, and meat quality of broilers could be due to its antioxidant and antibacterial properties. The improvement in the growth performance of broiler chickens caused by thyme could be partly attributed to its positive effect on nutrient digestibility. Furthermore, this positive result could be, at least in part, due to the antioxidant and antibacterial effects of herbal products in the gut 21.

Moreover, the antioxidant activity of O. majorana and related plants belonging to the Lamiaceae family has been widely investigated. Amyloid β protein-induced oxidative cell death is reduced by ursolic acid from Origanum majorana 22. Oregano essential oil and the ethanolic extracts exhibited the same antioxidant activity as α-tocopherol 23. Furthermore, the free radical scavenger effect of essential oil, alcoholic and aqueous extracts from O. majorana has been reported by many authors 24 25 .

Thyme oil and thyme powder have been reported to prevent liver damage by lowering lipid peroxidation levels 26. Additionally, thyme extract, rich in antioxidants, flavonoids, and polyphenols, has been shown to mitigate anxiety-like behaviors in animal models 27. Thyme oil protects against H_2O_2-induced cardiotoxicity by reducing lipid peroxidation and enhancing antioxidant activity 28 (Guesmi et al., 2020). In a study evaluating the effects of thyme oil and rosemary on osteoporosis, thyme oil exhibited greater bone-forming and anti-inflammatory properties than rosemary 29 .

Phenolic compounds are potent antioxidants found in many plants and herbs, including Thymus vulgaris. These compounds can react with membrane phospholipid bilayers to peroxidation chain reactions initiated by ROS. Thymol, the major Thymus vulgaris component, exhibits hepatoprotective properties 30. The anti-inflammatory properties of thymus essential oil are at least partially responsible for its hepatoprotective effects 31 .

Furthermore, gentamicin administration at high doses confirms this association. GM caused dyslipidemia, which is considered a major risk factor to CVD. Also, it is well documented that GM can induce renal damage. Thus, GM may have dual effects that could influence the induction of CVD. In our study, this was shown by the marked elevation in the atherogenic index in the GM positive control group compared with the negative control group 32. The Aqueous and alcoholic extracts of Origanum majorana and its volatile oil have been reported to demonstrate hepatoprotection against lead-induced hepatotoxicity. The alcoholic extract of O. majorana was more effective than its volatile oil or aqueous extract in protecting against lead-induced genotoxicity. We suggest the use of O. majorana extracts for populations exposed to low levels of lead 33.

Thyme essential oil may exhibit anti-carcinogenic effects due to the antioxidant activity of its constituents. Conversely, both mRNA and protein levels of Bcl-2 were elevated by hypoxia, while the phenolic isomer of thymol, carvacrol, at a

concentration of 600 microM, inhibited Bcl-2 expression in human pulmonary artery smooth muscle cells (PASMCs) under hypoxia 34 .

Histological results:

Microscopically, kidneys of rats from group 1 revealed the normal histological structure of renal parenchyma (Figs. 1 & 2). On contrary, kidneys from group 2 showed marked cytoplasmic vacuolization of epithelial lining renal tubules (Fig. 3 & 4), focal renal haemorrhage (Fig. 4) and congestion of glomerular tuft (Figs. 3 & 4). However, examined sections from group 7 revealed slight cytoplasmic vacuolization of epithelial lining some renal tubules (Fig. 5) and slight congestion of glomerular tuft (Figs. 5 & 6). Moreover, kidneys of rats from group 8 showed slight cytoplasmic vacuolization (Fig. 7) and necrobiotic changes (Fig. 8) of epithelial lining some renal tubules. On the other hand, kidneys of rats from group 9 showed no histopathological alterations (Fig. 9) except slight congestion of glomerular tufts (Fig. 10) in some examined sections.

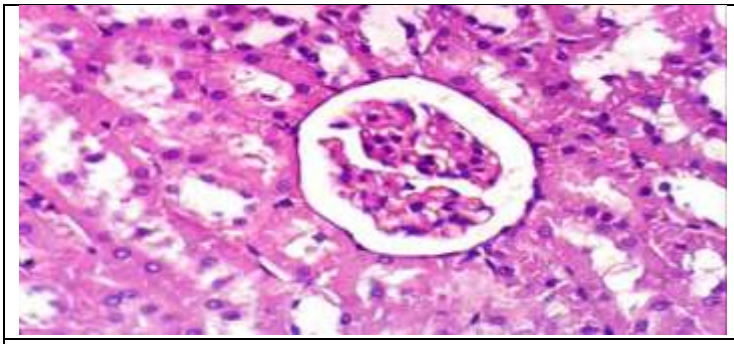

Fig. (1): Kidney of rat from group 1 showing the normal histological structure of renal parenchyma (H & E X 400).

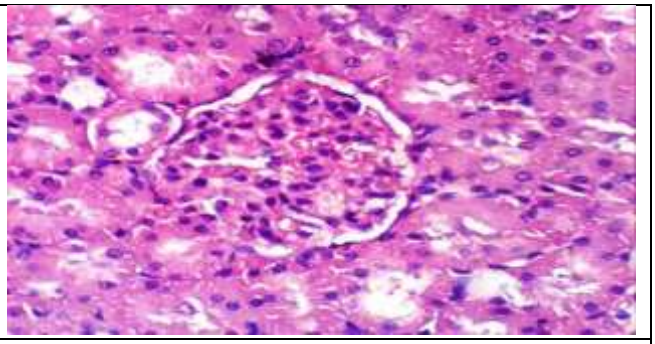

Fig. (2): Kidney of rat from group 1 showing the normal histological structure of renal parenchyma (H & E X 400).

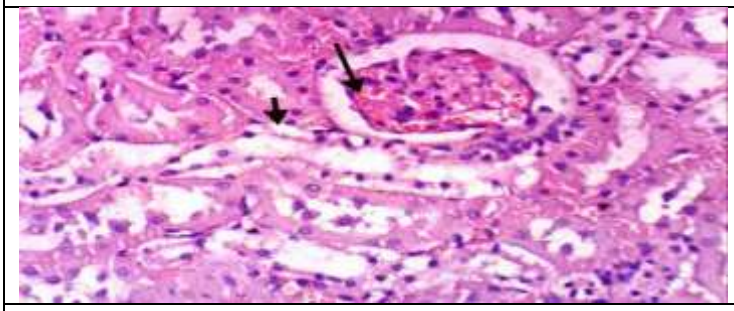

Fig. (3): Kidney of rat from group 2 showing marked cytoplasmic vacuolization of epithelial lining renal tubules and congestion of glomerular tuft (H & E X 400).

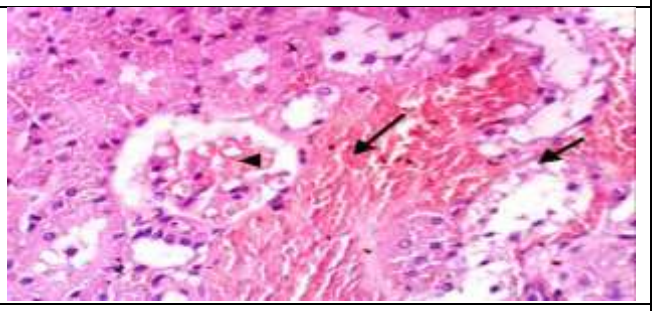

Fig. (4): Kidney of rat from group 2 showing marked cytoplasmic vacuolization of epithelial lining renal tubules, focal renal haemorrhage and congestion of glomerular tuft (H & E X 400).

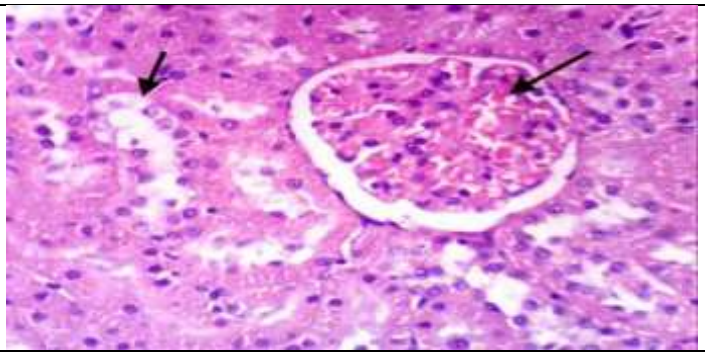

Fig. (5): Kidney of rat from group 3 showing slight cytoplasmic vacuolization of epithelial lining some renal tubules and slight congestion of glomerular tuft (H & E X 400).

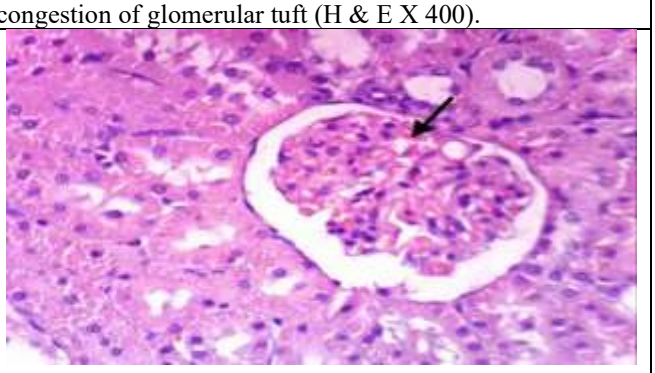

Fig. (6): Kidney of rat from group 3 showing slight congestion of glomerular tuft (H & E X 400).

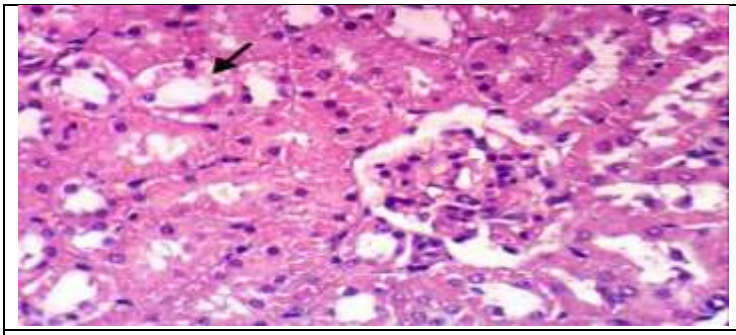

Fig. (7): Kidney of rat from group 4 showing slight cytoplasmic vacuolization of epithelial lining some renal tubules (H & E X 400).

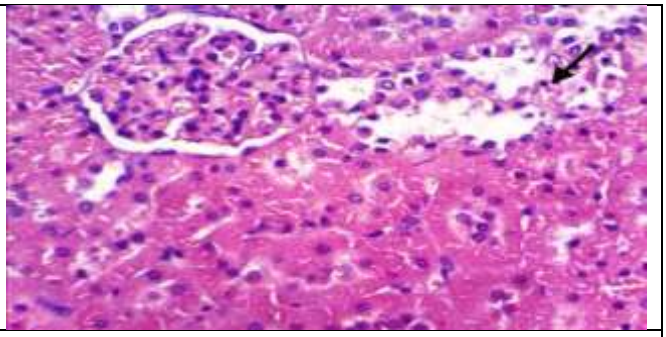

Fig. (8): Kidney of rat from group 4 showing slight necrobiotic changes of epithelial lining some renal tubules (H & E X 400).

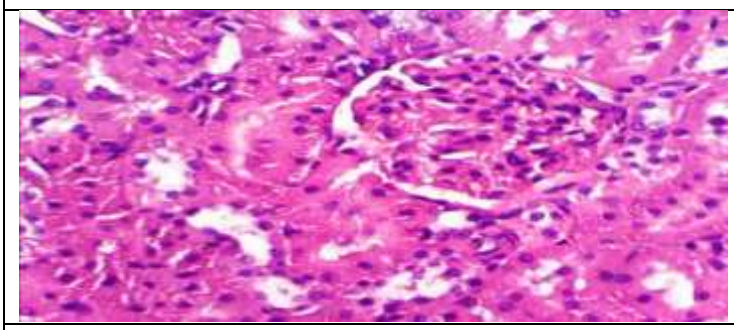

Fig. (9): Kidney of rat from group 5 showing no histopathological alterations (H & E X 400).

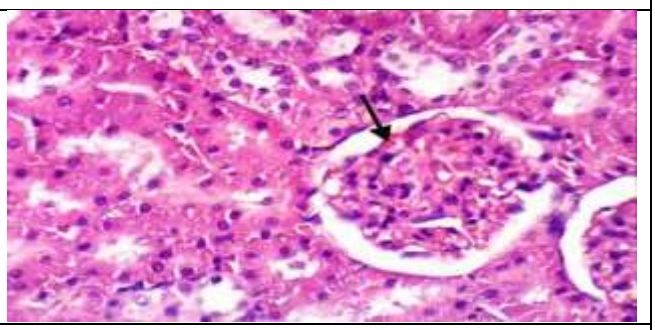

Fig. (10): Kidney of rat from group 5 showing slight congestion of glomerular tuft (H & E X 400).

CONCLUSION

In summary this study revealed that was proven that marjoram and thyme oils could be used as treatment agents for gentamycin-induced nephrotoxicity in rats indicating more effectiveness for thyme oil.

RECOMMENDATIONS

1. Marjoram and thyme oils could be used as treatment agents for gentamycin-induced nephrotoxicity as natural plants with little or no side effects.
2. Marjoram and thyme oils could be used to enhance kidney functions.
3. Further studies to make trials for different use of Marjoram and thyme oils with different doses and chronic diseases.

Significant Statement

Marjoram and thyme oils could be used as treatment agents for gentamycin-induced nephrotoxicity.

Conflicts of Interest

The author has no conflicts of interest to declare.

Author Contributions

M.A.(Marwa A. Ahmed): Study design, methodology, supervision, data analysis, result interpretation, manuscript writing & editing.

Data Availability

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

The author obtained ethical approval from the Agriculture Research Center – The Institutional Animal Care and Use Committee (ARC-IACUC) No (ARC-MU-81-25) - Ministry of Agriculture and Land Reclamation- Cairo- Egypt.

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