



[Science Archives \(ISSN:2582-6697\)](http://www.sciencearchives.org)

Journal homepage: www.sciencearchives.org



<https://doi.org/10.47587/SA.2022.3309>

Research Article



Estimation of flavonoids and tannins extracted from medicinal plants on gentamicin-induced nephrotoxicity in local Iraqi rabbits

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Received: July 30, 2022/ Revised: Aug 19, 2022/ Accepted: Aug 23, 2022

Abstract

The present study aimed to find out the effect The study aimed to extract flavonoids and tannin from sumac, myrtle, and on domestic rabbits exposed to nephrotoxicity with gentamicin. the percentage of flavonoids recorded from myrtle and Sumac is 13.8%, and 12.7%, respectively. The percentage of tannin recorded from myrtle and Sumac is 15.9 %, and 12.7%, respectively. The study was conducted inside the body of the organism and nephrotoxicity was developed with gentamicin by injecting animals 80 mg/ml/day (IP) for two weeks, after which I was dosed orally with the Flavonoid extract of sumac and myrtle and tannin extract of sumac and myrtle for a month at a rate of 1 ml/kg/day. A significant increase in the group's dose was found with gentamicin. An increase of 0.05 compared to the healthy control group. As well as a significant decrease of 0.05 for the groups treated with extracts compared to the control group for urea and creatinine. The total protein showed a significant increase of 0.05 for the group administered with gentamicin compared with the healthy control group and a significant decrease for the groups fed with extracts compared to the control group treated with gentamicin. As for albumin, a significant decrease was recorded for the group treated with gentamicin compared to the healthy control group by 0.05, and a significant increase for the groups treated with plant extracts compared with the control group treated with gentamicin. Uric acid showed a significant decrease in the groups treated with gentamicin compared with the healthy control group.

Keywords: Flavonoids, Tannins, Gentamicin, Biochemicals, Histological Study

Introduction

Herbs and spices have a long history in the therapeutic uses of humans, as many ancient texts have been found referring to their use in the Babylonian, Sumerian and Egyptian reference.

A small percentage of these plants, up to (1-10%), are used as food for humans and animals, which may possess some therapeutic properties that are due to their containing many primary and secondary metabolic active ingredients such as flavonoids, proteins, carbohydrates, alkaloids, minerals, oils, fats, polyphenols, tannins, carotenoids, terpenes and vitamins (Olowokudejo et al., 2008). Among these medicinal plants are sumac and myrtle. The sumac plant is spread in Iraq in mountainous regions (Alwan et al., 2009) and it contains a wide range of chemicals of nutritional and medicinal

importance. The proportion of sumac components varies according to its type and source (Al-Maadid, 2014). It contains phenolic compounds and terpenes and contains Fisetin, which is a bioflavins and contains Calic acid, its percentage reaches 11%, and the leaves, fruits, and coat of sumac contain tannin ranging from (20-35%) and it contains flavonoids up to 20% (Chhttopadhyay, 1996).

The sumac peel and pulp are characterized by containing phenolic compounds and many bioactive compounds, including flavonoids and tannin derivatives, and up to 4% of the fruit components, as well as gallic acid is the main active ingredient in its fruit extract. As for myrtle, it is one of the evergreen trees. It was used in the past for medicinal and food purposes because its leaves contain tannin and flavonoids,

such as quercetin, catechin, myricetin derivatives and volatile oils (AL-Anbori et al., 2007; Al-Salami, 2000).

Material and Methods

1. Selection of medicinal plants: Sumac seeds and myrtle leaves were chosen in this study due to their antioxidants and popular importance.

2. Collection of plant materials: Sumac seeds were obtained from local lawns in the city of Samarra, cleaned from dust, grinded, and preserved in dark, tightly sealed bottles until use. As for the myrtle leaves, they were collected from gardens and washed, dried, grinded and preserved in dark, sealed bottles until use. Classification of sumac seeds and myrtle leaves in the national Herbarium.

3. Preparation of extracts: Ten grams of Grinded and dry raw material (sumac seeds and myrtle both separately) were mixed with 100 ml of each of distilled water, ethanol, (both separately) in a 500 cm³ glass beaker and left for 24 hours at room temperature, after which the mixture was filtered. Using filter paper, a filtrate was obtained, and the preliminary assays were made on it.

4. Preliminary assay: Primary assays of the secondary metabolites were carried out by using tests on extracts to identify the active groups present in each extract.

As follows:

Detection of flavonoids (Sofowara, 2000)

The following tests were used to detect the presence of flavonoids in the extracts: Shinoda's test, Ferric chloride test, Alkaline's test, Lead - acetate test

Detection of phenolic compound and tannin (Kokate et al., 1993)

Ferric chloride test, Gelatin test, Lead-acetate test, Dichromate test

Extraction of flavonoids and tannins

Extraction of flavonoids: 10 grams of Grinded and dry plant sample were weighed, 100 ml of methanol were added to it and left for 24 hours, then filtered by a type filter paper. NO 42 Whatman, the filtrate was evaporated and then the sediment was dried in a drying oven and the weight was calculated. Which represents weight flavonoids in sample (Al-Samurai, 2014).

Extraction of tannin

Plant was naturally dried on trays away from sunlight at room temperature. The dry weight of the peel was measured and

powdered to obtain particles using an 80 mesh size (177 µm mesh size). Ten grams of the plant was added to 200 mL of 70% acetone aqueous solution in a 250-mL conical flask and was heated in a water bath for 3 h. The extract was filtered through qualitative filter paper (10–15 µm) to remove residues, and the final volume of solution was readjusted to match the starting volume. The aqueous extract was the plant tannin solution, which was stored at 4°C until used for follow-up studies. When the plant tannin solution was employed to remove *M. aeruginosa*, the plant tannin solution needs to dilute firstly, and the steps are as follows: 2 mL of the plant tannin solution was diluted to 30 ml. The diluted plant tannin solution was the final plant tannin flocculant (Atia et al., 2018).

In vivo study

Experiment design

The Sample Extract		Group Name	Dose
Myrtle	Extract Tannin	G1	80 mg / kg / day of gentamicin I.P for 14 days and then dosed with an extract orally 1 ml / kg / day for a month 80 mg / kg / day of gentamicin I.P for 14 days and then dosed with an extract orally 1 ml / kg / day for a month
	Flavonoid extract	G2	
Sumac	Tannin extract	G3	
	Flavonoid extract	G4	
C1 = the healthy control group was given water and diet C2 = negative control dosed with gentamicin 80mg/ kg / day I.P for 14 days (Salih@etal) ¹²			

After the dosing period ended, the animals starved for a period of hours (Riyam et al., 2020). 5 ml of blood were withdrawn by heart stab using a disposable medical syringe. They were discharged into clean, dry plastic tubes (disposable) free of anti-coagulants. Removing the serum by centrifuging the samples and using a centrifuge at a speed of 2500 rpm for 15 minutes and the serum was divided into four parts in small tubes (Eppendorf tube) and stored at -20° While the biochemical tests are under study, which are urea, creatinine, total protein, albumin, globulin, and uric acid.

Biochemical tests: The concentration of total protein, urea, creatinine, albumin and uric acid was were estimated by ready Kits from Bio Maghreb.

As for globulin, it was found through equation (Abdullah, 2009) Globulin = Total protein -albumin

Results and discussion

Colorimetric and inferential tests of extracts.

Table. 1 shows indicative tests for secondary metabolites in the aqueous and alcoholic extracts of myrtle leaves and sumac seeds

Plant	Detection	Test	Aqueous extract	alcoholic extract
Sumac	flavonoids	Shindo	-	-
		Ferric chloride	+	+
		Lead acetate	+	+
	Tannin	Alkaline solution	+	+
		Ferric chloride	-	+
		Potassium dichromate	+	+
Myrtle	flavonoids	Base lead acetate	+	+
		Gelatin	+	-
		Shindo	+	+
	Tannin	Ferric chloride	+	-
		Potassium dichromate	+	-
		Base lead acetate	+	+
		Gelatin	-	-

(+) Positive test: evidence of the presence of the chemical compound

(-) Negative test: evidence of the absence of the chemical compound

It is evident from the above table that the aqueous extract of sumac fruits contains flavonoids, which are considered antioxidants that contribute to suppressing free radicals and fight diseases, and this is consistent with what was mentioned (Boden et al., 1996), where among the last two sumac contains coumarin, which is part of the phenolic compounds

Also the aqueous extract of sumac contains tannin, and the results are consistent with (Boden et al., 1996), while the results for the aqueous and alcoholic extract of sumac do not agree with what is mentioned (Al-Hamdani, 2017; Loo and Bruyn, 1998).

The aqueous and alcoholic extract of myrtle contains flavonoids and tannin, which is consistent with this

Quantitative estimates of flavonoids and tannins in the study samples

Table 2 shows the percentage of extracted flavonoids and tannin from sumac fruits and myrtle leaf

The plant	% flavonoids	% tannin
Sumac seeds	14.51%	12.7%
Myrtle leaves	13.8%	15.9%

The results do not agree with what was reached (Shihab et al., 2016) as it was found that the percentage of tannin in sumac seeds is 11.5%, and the reason for the difference is due to the method of work followed, as well as the current result of tannin in the ace does not agree with (El Sissi et al., 1967), where it was stated that the percentage of tannin is 14.6%.

The percentage of flavonoids isolated from Myrtle, as well as for tannin for gram, with what is mentioned (Yanguie et al., 2021), where the latter mentioned that the percentage of flavonoids is 32% and water soluble tannin is 83.77%. The results of the current study agree with the findings of (Sakhr, Al-Khatib, 2020) that a high percentage of tannin is low, where the percentage is estimated at about 20% of the fruit mass, followed by the flavonoids.

Biochemical tests

Table 3 shows the mean ± standard deviation of the studied parameters

A total of 50 animals were used in the experiment, and they were divided into groups.

Result in Table 3 showed mean ± standard deviation of the parameters studied, it is evident that there is a significant increase in the C2 compared to the C1 group and this increase is consistent with Al-Obaidi, 2012 as well as the reason is due to the release of free radicals from the mitochondria of Renal tubules cell are the main factor of nephrotoxicity by gentamicin.

The results showed a significant increase in the levels of urea and creatinine concentrations in the C2 group dosed with gentamicin compared to the results of (Al-Majed et al., 2002) in the healthy control group. And the participation of toxic oxidative stress to the liver and kidneys resulting from gentamicin treatment and affects various metabolic pathways, thus increasing urea, creatinine, liver enzymes and uric acid.

Kidney function is evaluated by the level of concentration of urea, creatinine and uric acid in the blood It was found that gentamicin raises these levels in the blood serum, but if these levels are decreased in the kidneys, it indicates the presence of weakness in the kidneys. The reason for the weakness is the decrease in the glomerular filtration rate caused by the use of the drugs Aminoclycoside to which gentamicin belongs (Abdel-Raheem et al., 2010; Bartosikova et al., 2003).

Table 3. The mean ± SD of total protein, Albumin, Uric acid, Urea, Creatinine and for groups under investigation

Parameter	Mean±SD N=50						P- Value
	The Group = (mg/dl)						
	C1	C2	G1	G2	G3	G4	
Urea	24.2±2.8	37.06±5.26	28.02±2.51	35.26±2.5	28.99±5.19	33.9±15.6	0.05
Creatinin	0.8654±0.17	1.23±0.2	0.6748±0.1338	0.6740±0.509	0.6158±0.1193	0.455±0.277	0.05
Total Protein	7.2±0.62	4.302±0.877	4.842±1.551	6.188±1.081	6.95±0.645	5.482±0.345	0.05
Uric Acid	0.61±0.13	0.57±0.128	0.4800±0.1672	0.5003±0.1086	0.4254±0.1539	0.4224±0.05	0.05
Albumine	4.5±0.56	2.866±0.407	3.560±0.527	3.170±0.504	3.473±0.178	3.088±0.132	0.05
Glub	2.7	1.5	1.28	2.26	3.48	2.4	0.05

It is also due to the effect of gentamicin, which is responsible for tubular necrosis, destruction and death of epithelial cells, and leads to renal deficiency (Bartosikova et al., 2003) as well as the cause of the increase in the level of urea and creatinine to the loss of the direct energy source of glucose, forcing the animals to use alternative sources of energy such as fats and proteins, whose metabolism produces quantities of Urea as a byproduct (Oloyeda O.I, 2009). The results showed a significant decrease in the groups treated with flavonoid extract, tannin from sumac, flavonoids and tannin isolated from myrtle, compared with the control group dosed with gentamicin. It reduces the production of urea in the body, as well as the reason for the decrease due to the effectiveness of phenolic substances that have the ability to reduce oxidative damage of cells and glomeruli by sweeping free radicals and being an emulsifier of Metalchelatars, reducing agents, hydrogen-giving, and single oxygen radical suppression agents (Abdel-Raheem et al., 2010).

We notice through the current study a significant increase in the amount of protein for groups treated with plant extracts, due to the high role of phenolic compounds such as Phenolic acid and flavonoids in enhancing cellular defenses against oxidation, reducing the process of oxidation of proteins and activating antioxidants such as Catalase, SOD and GPX, in addition to non-enzymatic substances. Like glutathione, it is attributed to the role of phenolic compounds and poly flavonoids in enhancing the production of glutathione and reducing the process of lipid peroxidation and oxidation of protein substances, and this leads to maintaining the level of total protein concentration (Abdel-Raheem et al., 2010). The

increase in the level of total proteins is attributed to the active compounds in (sumac and myrtle) that work to remove the destructive toxic effect that they can have on the renal tissues or by re-repairing the damaged ones, thus preventing the loss of proteins, which causes an increase in its concentration in the blood serum, or Multiple phenolic compounds may reduce oxidative damage and its effect on kidney cells and glomeruli, and this reduces the filtration of proteins from the blood and their excretion with the urine (Bartosikova et al., 2009).

The results showed a significant decrease in the albumin level in the C2 when compared to the C1 group. Albumin filtered from the blood into the urine through the renal glomeruli (Priya et al., 2011).

The results of the current study showed a significant increase in the level of uric acid in the control group treated with gentamicin compared to the non-dose control and this result is consistent with the findings of Nidhal AK et al., 2012, which showed a significant increase in the level of uric acid in the serum of rats dosed with gentamicin.

The current study showed a significant decrease between the groups treated with flavonoid extract and tannin for sumac and myrtle and between the control group dosed with gentamicin. The reason for the decrease was due to the use of flavonoids in the plant that help in the treatment of pathological conditions associated with high uric acid, and the flavonoids present in many plants showed an inhibitory effect of the (Xanine oxidase) XO enzyme. For uric acid (Pavia et al., 2009; Haidari et al., 2008).

Histological study

Effects on the histology of the kidney

Group 1: The kidney of adult male rabbits appeared control section of infected groups: congestion of blood vessel, desquamation of thin segmented loop of henle from renal interstitium, fibrinoid in the lumen of papillary ducts.

Group 2: The kidney of Tannin extracted from myrtle desquamation with degenerated epithelial layer of papillary ducts, Ghost epithelial layer with pyknotic of nuclei papillary ducts), fibrinoid within lumen of papillary ducts.

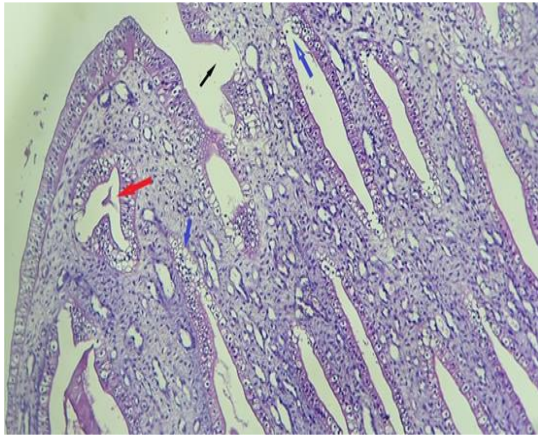


Fig.1 The kidney control section of infected groups: congestion of blood vessel (black arrow), desquamation of thin segmented loop of henle from renal interstitium (yellow arrow), fibrinoid in the lumen of papillary ducts 40X: H&E.

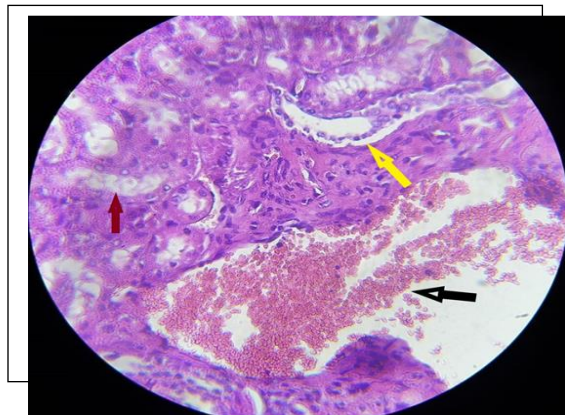


Fig. 2 desquamation with degenerated epithelial layer of papillary ducts (black arrow), Ghost epithelial layer with pyknotic of nuclei papillary ducts (blue arrow), fibrinoid within lumen of papillary ducts (40X H&E)

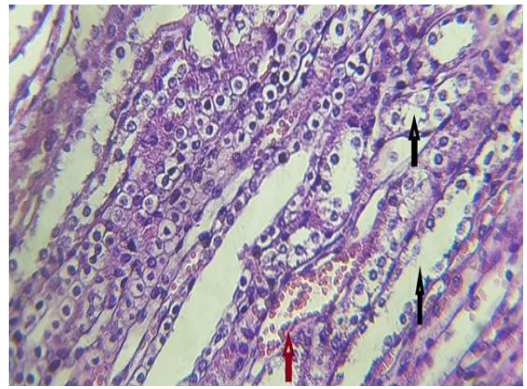


Fig. 3 extracted flavonoids from myrtle. Ghost epithelial cell layer thick segment of the loop of Henle (black arrow), congestion of blood vessel (red arrow) (40X: H&E).

Group 3: The kidney of extracted flavonoids from myrtle desquamation with degenerated epithelial layer of papillary ducts, Ghost epithelial layer with pyknotic of nuclei papillary ducts), fibrinoid within lumen of papillary ducts.

Group 4: The kidney of extracted Tannin from sumac Tannin normal epithelial layer of thick segment of the henle loop.

Group 5: The kidney of sumac extracted flavonoids congestion of blood vessel, degeneration in epithelial layer of papillary ducts (40X : H&E).

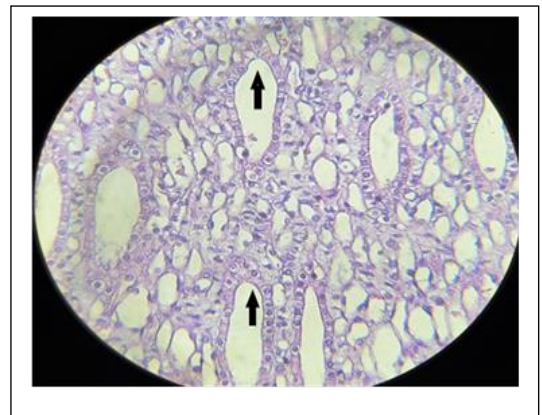


Fig. 4 Normal epithelial layer of thick segment of the henle loop (black arrow) (40X : H&E)

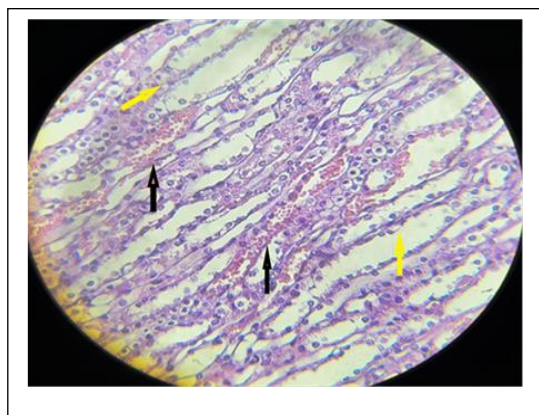


Fig. 5 extracted flavonoids from sumac congestion of blood vessel (black arrow), degeneration in epithelial layer of papillary ducts (yellow arrow) (40X : H&E)

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication

Ethical Considerations

The study was approved by the institutional ethical committee

Funding support

The author declares that they have no funding support for this study

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How to cite this article

Hadi, N. A., Ahmed, A. S., Rabeah, T., Ali, M. H. (2022). Estimation of flavonoids and tannins extracted from medicinal plants on gentamicin-induced nephrotoxicity in local Iraqi rabbits. *Science Archives*, Vol 3(3), 204-210. <https://doi.org/10.47587/SA.2022.3309>

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