Effect of Ruta Graveolens Extract on Hepato and Nephrotoxicity Induced by Gentamicin on Experimental Rats

Ttasneem Sobhy Fahmy and Asmaa Ahmed M. Hussein

Nutrition and food science dept. Home Economics, Helwan University
tasnem_sobhy@yahoo.com

Abstract

This study was carried out to investigate the effect of ruta graveolens extract on hepato and nephrotoxicity induced by gentamicin on experimental rats. 35 albino rats of Sprague-Dawley strain were randomly distributed after adaptation period into two main groups (7 of each), the first main group was kept on the basal diet as negative control (-ve), The rest of the animals (n = 28) were subcutaneously injected with gentamicin (100ml/ kg body weight/ 7day/i.p) for the induction of hepatotoxicity and nephrotoxicity .After injection rats were divided into 4 subgroups. The first subgroup of rats was as a positive control group (+ve). The other subgroups were fed on the experimental diets containing different levels of ruta graveolens extract 5%, 10% and 15%, respectively. At the end of the experimental period (6 weeks) liver and kidneys functions were determined. Also, antio xidants enzymes were determined. Histopathological examinations for liver were done. The results indicated that liver enzymes ALT, AST and ALP were decreased in groups 3, 4 and 5, respectively compared with the positive control group. Uric acid and creatinine were significantly improved in all tested groups compared with the positive control one. Moreover, antioxidant enzymes (GSH, SOD and CAT) were significantly improved in all examined groups compared with the positive control group. It could conclude that Ruta Graveolens had a potential effect against on hepato and nephrotoxicity damage therefore this study recommends increasing dietary intake of Ruta Graveolens could be beneficial for patients with hepatic and nephrotoxicity damage.

Key words: Ruta Graveolens, liver enzymes, Hepatic Damage, Antioxidant Enzymes.

Introduction

Ruta graveolens L. (Fetid Street) is a perennial herb with a woody stem, branching from the base, smooth and round, up to 80 cm high, belonging to the family Rutaceae. The main active substances of the medicinal plant Ruta graveolens are flavonoids (quercetin; rutin), furocoumarins and alkaloids (Kathirvel et al., 2017). Rutin, which is a phytochemical compound, has already shown its multiple pharmacological benefits including antioxidant, neuroprotective, cardioprotective, or even anti carcinogenic effects (Camille et al., 2023).

Renal physiology plays an important role in maintaining homeostasis by regulating; the water and acid-base balance, electrolyte composition, blood

pressure, erythropoiesis, and production of some enzymes and hormones (Puelles & Huber , 2022). Its main function is the excretion of potentially harmful substances including drugs, infectious agents, and toxic ants from the body (Gilbert &Weiner, 2022) Thus, nephrotoxicity largely consists of renal injuries caused either directly or indirectly from medications that clinically present with glomerulopathies and tubulopathies Sales & Foresto, (2020).

Nephrotoxicity is the major dose-limiting side effect of many chemotherapeutic including cisplatin and doxorubicin therapy (Oun et al., 2018) The gold standard in practice to confirm clinical nephrotoxicity includes elevated serum creatinine, uric acid, sodium, potassium, calcium, ATPase levels and blood urea nitrogen while the same parameters are decreased in urine samples. The elevation of prominent biomarkers in serum and not in urine could be due to tubular obstruction and back-leakage of substances in the renal tubules leading to deficient protein synthesis, membrane lipid per oxidation Ranasinghe et al., (2023)

Liver disease accounts for approximately 2 million deaths per year worldwide, 1 million due to complications of cirrhosis and 1million due to viral hepatitis and hepatocellular carcinoma. Cirrhosis is currently the 11th most common cause of death globally and liver cancer is the 16th leading cause of death; combined, they account for 3.5% of all deaths worldwide. (Asrani et al., 2018). The same authors reported that approximately 2 billion adults are obese or overweight and over 400 million have diabetes; both of which are risk factors for non-alcoholic fatty liver disease and hepatocellular carcinoma.

Liver is one of the dynamic organs that has a crucial role in macromolecules metabolism, bile acid biosynthesis and detoxification process of circulating agents (Zheng et al., 2022). Liver injury is attributed to multiple issues as subjection to repeated doses of drugs, medicine-based treatment, and toxic substances in the surrounding environment (Attallah et al., 2022). Gentamicin can cause kidney problem and inner ear problems . The inner ear problems can include problems with hearing loss and balance. If used during pregnancy, it can cause harm to the developing fetus. However, it appears to be safe for use during breastfeeding. Gentamicin is a type of aminoglycoside and works by disrupting the ability of the bacteria to make proteins, which typically kills the bacteria. Gentamicin also, occurs as consequence of some diseases like non-insulin dependent diabetes mellitus, chronic kidney, and heart failure (Byrne and Targher, 2022). In many cases of untreated liver injury is progressed to liver cirrhosis, fibrosis, and cancer (Kermanizadeh et al., 2022).

Materials and methods

Materials:

Gentamicin, cellulose, vitamin mixture and minerals, kits for biochemical analysis were purchased from El-Gomhoria Company for Trading Drugs, Chemicals and Medical Requirements. Ruta graveolens were purchased from the local market.

Thirty-five albino rats of Sprague-Dawley strain weighing approximately 150 ± 5 gm were purchased from Helwan Farm for Experimental Animals, Cairo, Egypt.

Methods:

Experimental animal design:

Preparation of basal diet:

The basal diet was prepared according to ***Reeves et al. (1993)***. It consists of 20 % protein, 10 % sucrose, 4.7% corn oil, 2% choline chloride, 3.5 % salt mixture, 1% vitamin mixture, and 5% fibers. The remainder was corn starch up to 100 %.

Animals were divided into two main groups (n=7, once). The first main group (n=7) was fed on the basal diet during the experimental period as a negative control group (-ve). The rest of the animals (n=28) were subcutaneously injected with gentamicin (100ml/ kg body weight/ 7day/i.p) for the induction of hepatotoxicity and nephrotoxicity. This dosage was proved to be effective from the earlier report as stated by ***Adriana et al., (2022).***

After injection, animals were divided into 4 subgroups as follows:

Group1: Rats with hepatic and nephro toxicity were fed on the experimental diet as positive control group.

Group2: Rats with hepatic damage were fed on the experimental diet and receive 1ml/day of Ruta Graveolens extract at concentration 5 %.

Group3: Rats with hepatic damage were fed on the experimental diet and receive 1ml/day of Ruta Graveolens extract at concentration 10 %.

Group4: Rats with hepatic damage were fed on the experimental diet and receive 1ml/day of Ruta Graveolens extract at concentration 15 %.

At the end of the experiment (6 weeks) all rats fasted overnight, lightly anesthetized under ether. Blood was withdrawn into clean dry centrifuge plastic tubes. Blood samples were centrifuged and serum was obtained then stored at -20º C in a clean well stopped vial until analysis.

Biochemical Analysis:

The liver enzyme alanine aminotransferase (ALT) was determined in serum to the method of ***Sherwin, (1984).*** Aspartate aminotransferase (AST) was determined according to ***Young, (1990).*** Serum alkaline phosphatase

(ALP) was determined according to the method described by ***Roy, (1970).*** Serum uric Acid concentration was determined by the method of ***Fossati et al., (1980)***. Creatinine was determined according to the method described by ***Henry, (1974)***. Serum glutathione (GSH) was determined according to **Carlberg and Mannervik, (1985).** Serum CAT activity was measured in tissue homogenate according to ***Aebi, (1984).*** Serum superoxide dismutase (SOD) was determined according to **Aebi,** (**1984),** and **Nishikimi et al., (1972)**, Serum triacylglycerols was determined enzymaticlly according to the method described by **Fassati and Perncipe, (1982).** Serum total Cholesterol was enzymaticlly determined according to the method described by **Ellefson and Caraway, (1976).** Serum HDL-C was determined calorimetrically according to the method described by **Lopez-Virella *et al.,* (1977).** Serum LDL-C was calculated according to Friedewald's formula **(Friedewald *et al.,* 1972)**.

Statistical analysis:

Autopsy samples were taken from the liver of rats in different groups and fixed in 10% formalin solution. The results were expressed as mean ± standard error (SE). The statistical analysis was carried out by using SPSS, PC statistical software (Verion 18.0 SPSS Inc., Chieago, USA) using the Dunk 'test multiple range post-hoc test. Data were analyzed by one way analysis variance (ANOVA). The values were considered significantly different at P <0.05 **(*Snedecor and Cochran, 1980*)**.

Results and Discussion

Recorded results in Table (1) showed the effect of supplemented diet with ruta graveolens extract on the activity of ALT& AST and ALP in rats with hepatotoxicity and nephrotoxicity. It showed that the injected rats with gentamicin alone without adding Ruta graveolens extract (positive control group) have a significant increase in the serum levels of ALT& AST and ALP, compared to normal rates. Whilst the treatment of hepatotoxicity rats by feeding on a supplemented diet with 5, 10 and 15% of Ruta Graveolens extract caused reduce significant in the serum concentration of ALT& AST and ALP, compared to untreated hepatotoxicity rats. The best improved results were reported in rats treated with high levels (15%) of Ruta Graveolens extract.

Gentamicin is naturally produced by the bacterium Micromonospora purpurea,was patented in 1962, approved for medical use in 1964.The antibiotic is collected from the culture of the Micromonospora by perforating the cell wall of the bacterium. Current research is underway to understand the biosynthesis of this antibiotic in an attempt to increase expression and force secretion of gentamicin for higher titer. Gentamicin is on the World Health Organization's List of Essential Medicines.[11] The World Health

Organization classifies gentamicin as critically important for human medicine. It is available as a generic medication.

Ruta species are important because many active compounds and ruta have many different secondary metabolites such as flavonoids have been isolated and identified from them. A number of chemical constituents such as alkaloids, coumarins, terpenoids, volatile substances, flavonoids and furoquinolines have been isolated from different parts of the plant **(kuzovkina et al., 2004).** The existence of saponin, Tannins and glycosides has also been proven **(Hashemi et al., 2011).** Quercetin and rutin are the main active flavonoids of R. graveolens. Rutin was first isolated from the leaves of R. graveolens. Ruta have a high content of aliphatic acids, alcohols and ketons were found in R. graveolens. R. graveolens produces high levels of linear furanocoumarins, mostly methoxypsoralen and psoralen. Quercetin and Rutin both are able to inhibit the hepatic aldehyde oxidase activity, which was in a dose dependent manner. The inhibitory effect of Quercetin on the enzyme was found to be more potent than menadione, the known specific inhibitor of aldehyde oxidase (**Gravot et al., 2004).** All this active component of R. graveolens enhanced the liver enzymes as shown in our results.R. graveolens extract contains bioactive compounds which, independently of known photo activatable mechanisms, potently inhibit cancer cell proliferation and survival through multiple targets.

Ruta graveolens extract prevent the replication and proliferation of DNA and mitosis.The apoptosis of hepatocytes and renal tubular cells were occurred in gouts treated with the extract of Ruta. Also cell death and apoptosis in colonic crypts were occurred in mice treated with Quercetin and Rutine prepared from Ruta graveolens. In a study similar to our study, had shown mild to moderate hepatotoxicity in laboratory findings using hydroalcoholic extract of Ruta in mice. Although dried Ruta graveolens is known as a plant with few complications commonly, they observed that the aqueous extract of it has a more detrimental effect than the ethanolic extract.

Kannan and Baba, (2012) recorded more than 120 different phytochemical compounds from R. graveolens root and aerial parts. only a significant elevation of alkaline phosphatase was noticed during treatment with R. graveolens **(Sahar *et al., 2022*)**.Ruta graveolens L. (Rutaceae) is a medicinal plant widely used in the Mediterranean region to treat pain, dermatitis, rheumatism, and other inflammatory diseases, but its use is limited by its potential toxicity .

Table (1): Effect of Supplemented Diet with Ruta Graveolens extract on the Activity of ALT& AST and ALP in the experimental Rats

Groups		Parameter as Mean ± SD		
		ALP (u/ml)	ALT (u/ml)	AST (u/ml)
Negative control group		2.06 ± 0.08 [e]	33.09 ±2.69 [e]	43.15 ± 1.54 [e]
Positive control group		8.75 ±0.79 [a]	101.39 ±7.12 [a]	124.99 ±3.81 [a]
Treated groups with Ruta Graveolens extract at concentration	**5%**	6.64 ±0.34 [b]	84.20 ±3.64 [b]	103.08 ± 5.70 [b]
	10%	5.41 ± 0.28 [c]	70.13 ±3.50 [c]	82.32 ± 3.65 [c]
	15%	4.49 ± 0.51 [d]	52.10 ± 3.28 [d]	66.58 ± 4.30 [d]

Values are expressed as mean ± S.D. Means with the different superscript letters in the same column were significantly different at $P \leq 0.05$.

The results showed in Table (2), explained that rats with hepatotoxicity and nephrotoxicity have an increased significant ($p<0.05$) in serum Cr and UA concentrations in injected rats of gentamicin and fed on a basal diet alone (positive group) (7.93 ±0.71&3.36±0.34) respectively, compared with normal rats (2.31 ± 0.28 &0.61±0.03) respectively, However, the results showed that there was a decrease significant in serum Cr and UA concentrations of injured groups gentamicin given the different levels of Ruta Graveolens extract, compared to the positive group. The best improvement in serum level of Cr and UA is shown in the treated groups by Ruta Graveolens extract at a level of 15% compared to the other treated levels. Ruta graveolens is endowed with potent anti-inflammatory effects due to the presence of bioactive compounds like flavonoids. Since inflammation is a hallmark of neurodegenerative diseases, Ruta graveolens has been considered as a potentially effective remedy in this field.

Treatment of chlorpyrifos- and methomyl-administered rats with S. officinalis and R. graveolens ethanolic leaf extracts significantly improved the elevated levels of creatinine, urea and uric acid towards the normal levels. These biochemical results are concomitant with the histological results which indicated that S. officinalis and R. graveolens ethanolic leaf extracts have the potentials of improving the histological architecture and integrity of the kidneys of chlorpyrifos- and methomyl-administered rats as sections obtained from this study showed a better histological organization of the kidneys in the extract treated rats **(Osama *et al.,* 2017).** These results are in concurrence with other publication which elucidated that the hydroalcoholic extract of S. Officinalis exhibited nephroprotective effect in gentamicin-induced renal damage probably by its anti-oxidant activity, but further studies

on its exact mechanism of action are wanted **(Ashtiani *et al.,* 2013). Mahmoud *et al.,* (2015)** demonstrated the protective effect of R. graveolens leaf extract against diethylnitrosamine-induced kidney damage in rats .

The phytochemical component of the R. graveolens has demonstrated the presence of flavonoids, rutin, quercetin, lemonins and furocoumarin as its major active constituents. Rutin and quercetin have been reported to possess anti-oxidant, anti-renal disorders, anti-inflammatory, anti-thrombotic and superoxide scavenger properties. Besides these, several essential oils have been reported in R. graveolens having anti-inflammatory, anti-renal disorders and cytotoxic properties (**Khori et al., 2008)**.Some people use ruta for breathing problems including pain and coughing due to swelling around the lungs (pleurisy). Rue is used for other painful conditions including headache, arthritis, cramps, and muscle spasms; and for nervous system problems including nervousness, epilepsy, multiple sclerosis, and Bell's palsy.

Table (2): Effect of Supplemented Diet with Ruta Graveolens extract on Serum Concentrations of Creatinine and Uric acid in the experimental Rats

Parameters / **Groups**		**Parameter as Mean ± SD**	
		Uric acid (mg/dl)	**Creatinine (mg/dl)**
Negative control group		0.61±0.03 [d]	2.31 ± 0.28 [d]
Positive control group		3.36±0.34 [a]	7.93 ±0.71 [a]
Treated groups with Ruta Graveolens extract at concentration	**5%**	2.24±0.29 [b]	6.25 ± 0.52 [b]
	10%	1.46±0.04 [c]	4.85 ± 0.27 [c]
	15%	1.11± 0.10 [c]	3.90 ± 0.14 [c]

Results in Table (3), explained the effect of the supplemented diet on the serum levels of total Cholesterol (TC), Triglyceride (TG), HDL-c and LDL-c. In comparison to the negative control group, IP injection of gentamicin induced a significant ($P<0.05$) increase in serum concentrations of TG, Cholesterol and LDL-c and decrease in the level of HDL-c. However, in comparison to the positive control group, when rats feeding on the supplemented diet with ruta graveolens extract resulted indicated significantly lower in serum levels of total Cholesterol, triglyceride, LDL-c and increase in the level of HDL-c. A better improvement in serum levels of TC, TG, HDL-c and LDL-c, was discovered in treated hepatotoxicity rats with the mixture of different levels of ruta graveolens extract.

Dana et al., (2020) Showed that there was an increase in HDL and a decrease in TG and LDL in rats fed ruta chalepensis L. This effect on blood lipid profile may be explained by the ability of plant fibers to reduce the cholesterol absorption, increase the clearance of TG and LDL and increase the release of HDL. This finding agrees with the results of **Makni et al., (2008)** who found a significant inverse relationship between lipid parameters and the fiber content. also found that rats fed a rich-cholesterol diet had an increase in plasma TG, TC and LDL levels, with decreased circulating HDL, thus providing a model for dietary hyperlipidemia. This result was consistent with those of the present study.

Toserkani et al., (2012) reported that cholesterol and LDL reduction using the plant extract, although were not observed any qualified changes in glucose, triglycerides, VLDL and HDL levels. They showed cholesterol and LDL reduction levels in diabetic rats after Rue extracts medication. **Mitra et al., (2019)** reported that using Ruta Graveolens and their flavonoids extracts caused cholesterol reduction in diabetic rats streptozotocin Ruta graveolans Flavonoids & streptozotocin Ruta graveolans total (SRF and SRT) in comparison with control (C) and treated Atorvastatin diabetic rats. These results confirmed that R. graveolens flavonoids extract has antitriglycerides effect in comparison with its total extract. Ruta Graveolens groups had respectively the most glucose, urea and cholesterol reductions levels. But total and flavonoids extracts and Metformin were not effective on triglycerides reduction. These results are similar to **Malika et al., (2017)** study which reported antilipidemic effects of Ruta Graveolens.

Table (3): Effect of Supplemented Diet with Ruta Grave lens extract on the Activity of Cholesterol & Triglycride & HDL and LDL in the experimental Rats

		Parameter as Mean ± SD			
		Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Negative control group		63.30 ±4.12 [d]	82.49 ± 2.98 [d]	36.82 ±1.30 [a]	14.50±0.74 [d]
Positive control group		115.00 ±4.15 [a]	136.65 ± 3.66 [a]	11.12 ±0.75 [e]	63.06± 3.01 [a]
Treated groups with Ruta Graveolens extract at concentration	5 %	91.37 ±3.81 [b]	119.80 ± 4.47 [b]	2.24 ± 2.00 [b]	41.99±2.01 [b]
	10 %	84.30 ±6.12 [b,c]	101.99 ± 2.97 [c]	1.46 ± 2.38 [c]	36.58±1.24 [b]
	15 %	77.74 ± 2.37 [c]	96.14 ± 2.87 [c]	1.11 ± 1.30 [c,d]	25.22±2.74 [c]

Results in Table (4) illustrate the effect of Ruta Graveolens extract on tissue liver lipid peroxidation and antioxidant enzymes of experimental rats.

Results of superoxide dismutase (SOD) concentration; as a marker of oxidative stress; hebetic control group showed a significant decrease in SOD concentration compared with the normal control group with a mean value of (0.91± 0.02 u/ml and 3.66±0.28 u/ml) respectively. Furthermore, the treatment of hepatotoxicity rats by feeding on a supplemented diet with 5, 10 and 15% of Ruta Graveolens extract caused reduce significant increase in resulted in SOD concentrations compared with the hebetic control group.

The data in the same table clarified that Glutathione (GSH) level was a decrease in the hebetic control group with a mean value of (1.16± 0.07 µ /mg) compared with the normal control group (5.75± 0.28 µ /mg). However, all treated groups showed a significant increase in GSH levels compared with the hebetic control group.

Concerning the first studied marker of oxidative stress; catalase (CAT) concentration; results showed that there was a decrease in CAT concentration of hebetic control group with a mean value of (1.09± 0.04 u/ml) compared with the Normal control group (4.44± 0.20 u/ml). All treated groups showed a significant increase in CAT concentration compared with the hebetic control group. The best improved results were reported in rats treated with high levels (15%) of Ruta Graveolens extract.

Mohammad *et al.,* (2024) illustrated that ruta extracts and Ruta-ND treated rats at the two doses displayed a significant reduction in the oxidative stress parameters compared to Ova-sensitized rat. Regarding MDA, there was a statistically significant reduction in the production of MDA in all of the treated groups**.** Regarding SOD level, all R. graveolens treated groups showed a significant increase in the SOD level compared to Ova-sensitized group. It was noted that there were no significant differences in MDA and SOD levels between Ruta 100 mg and 200 mg. These results indicate strong antioxidant properties of R. graveolens which was supported by previous studies **(Asgharian et al., 2020, Pawankar et al., 2015).** This strong antioxidant effect may be attributed to the high content of flavonoids, especially rutin and phenolic acids in R. graveolens **(Park et al., 2009).**

The major active R. graveolens components flavonoids, rutin, quercetin, furocoumarin and lemonins plays an important role and a “lead” as a natural substance in various diseases and anti-oxidant effect , anti-inflammatory conditions and for many synthetic drugs (**Khori et al., 2008)**.

Table (4): Effect of Supplemented Diet with Ruta Graveolens extract on the Serum Concentrations of MDA and the Activity of SOD Enzymes in the experimental Rats

Parameters / C		Parameter as Mean ± SD		
		GSH (m. mol/mg/ protein)	CAT (u/mg/ protein)	SOD (u/mg/ protein)
Negative control group		5.75± 0.28 [a]	4.44± 0.20 [a]	3.66±0.28 [a]
Positive control group		1.16± 0.07 [e]	1.09± 0.04 [d]	0.91± 0.02 [d]
Treated groups with Ruta Graveolens extract at concentration	5%	2.34±0.18 [d]	2.04± 0.10 [c]	1.73±0.25 [c]
	10%	3.07±0.15 [c]	2.82±0.13 [b]	2.55 ± 0.23 [b]
	15%	2.34±0.14 [b]	3.45±0.46 [b]	3.02 ± 0.10 [b]

Histopathological Examinations:

Histopathological examination of liver:

Microscopic examination of liver sections from the control group (**pho. 1**) revealed normal structure of hepatic parenchyma.

The liver sections from PC group (**Pho. 2**) revealed random multifocal inflammatory cells infiltration in the hepatic parenchyma. Excessive vacuolar degeneration of hepatocytes was observed in almost examined sections.

Widely grown in different parts of the world, this herb has historically been in use since the ancient times. Its documented therapeutic uses include the treatment of inflammatory conditions, liver problems, eczema, ulcers, arthritis, fibromyalgia, antidote for venoms, and insect repellent.

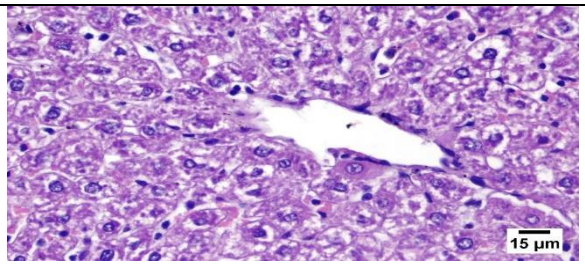
Limited improvement was observed in TTT 5% group (**Pho. 3**) that characterized by fewer portal inflammatory cells infiltration and edema in some examined sections and multifocal hepatic necrotic areas with fewer inflammatory cells aggregation. Similar results were observed in TTT 10 % with vacuolar degeneration in some instances (**Pho. 4**). Apparently normal hepatic was recorded in TTT 15% group (**Pho. 5**).

Histopathological examination of Kidney:

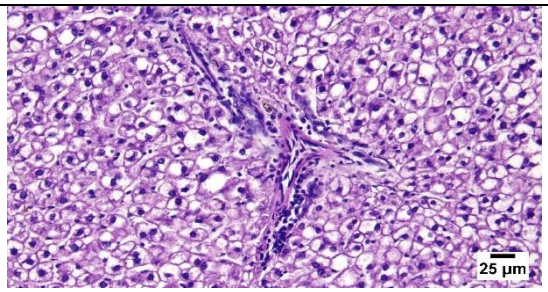
Histopathological examination of kidney sections from control group (**Pho. 1**) revealed normal renal cortex and medulla. Meanwhile, examination of PC revealed diffuse necrosis of the renal tubular epithelium associated with multifocal areas of interstitial nephritis (**Pho. 2**). Moderate improvement was detected in TTT 5% group that characterized by vacuolar degeneration in the tubular epithelium with multifocal interstitial nephritis (**Pho. 3**). Marked

improvement was observed in TTT 10% that revealed congestion of some blood vessels in the renal parenchyma with accumulation of eosinophilic renal casts in the tubular lumen in some instances (**Pho. 4**). Apparently normal renal parenchyma was observed in TTT 15 % in almost examined individuals (**Pho. 5**).

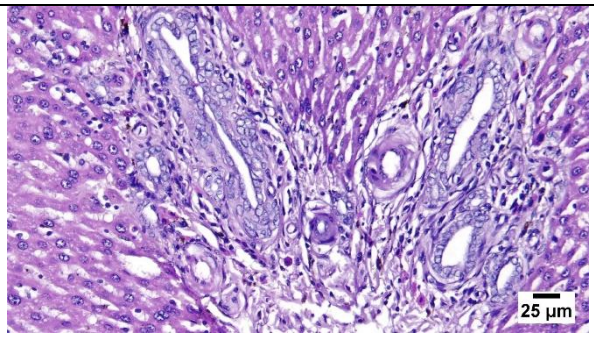
Histopathological examination of liver:



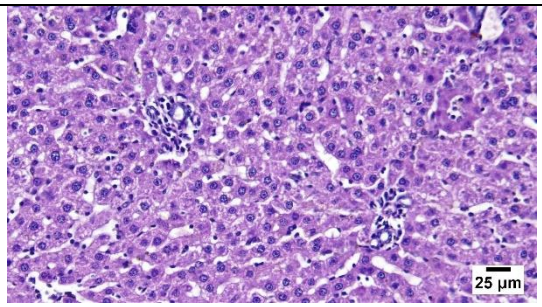
Pho. (1): Photomicrograph of liver, normal group higher magnification showing normal hepatic parenchyma (H&E).



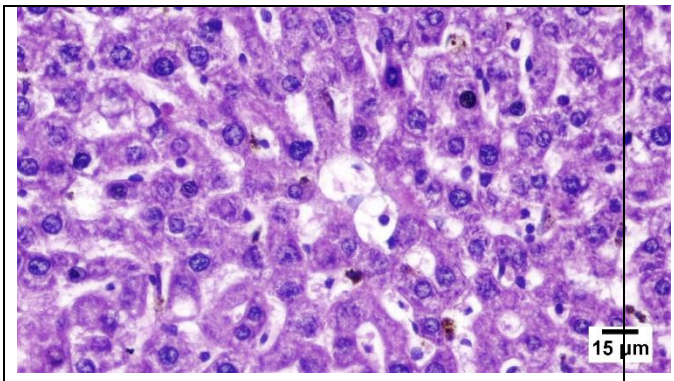
Pho. (2): Photomicrograph of liver, PC group showing portal mononuclear inflammatory cells infiltration (arrow) with vacuolar degeneration in the hepatocytes (H&E).



Pho. (3): Photomicrograph of liver, TTT 5% group showing mild portal fibroplasia with fewer inflammatory cells infiltration (H&E).

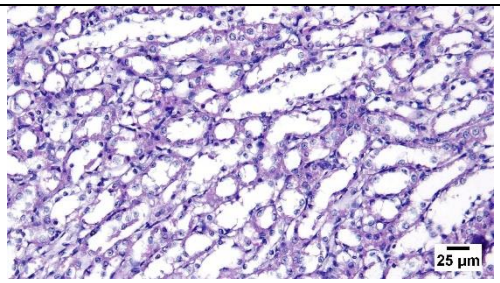


Pho. (4): Photomicrograph of liver, TTT 10% group showing mild portal inflammatory cells infiltration (H&E).

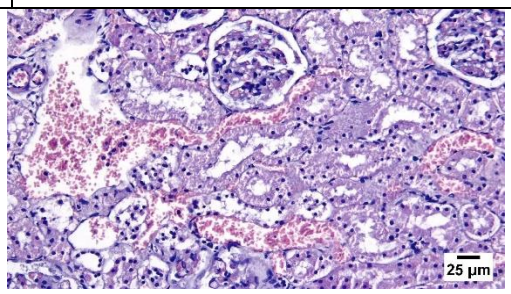


Pho. (5): Photomicrograph of liver, TTT 15% group showing apparently normal hepatic tissue (H&E).

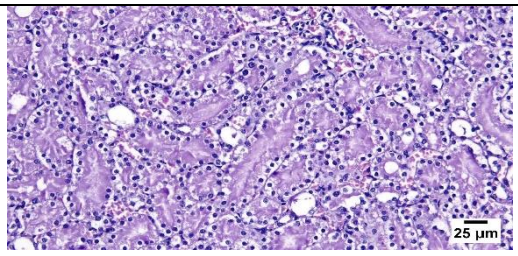
Histopathological examination of Kidney



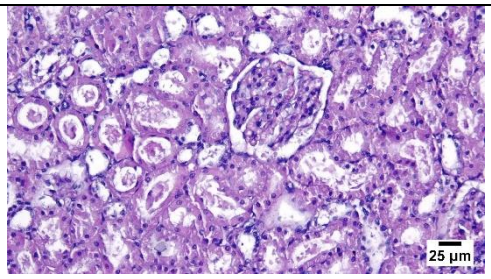
Pho. (1): Photomicrograph of kidney, control group showing normal renal medulla (H&E).



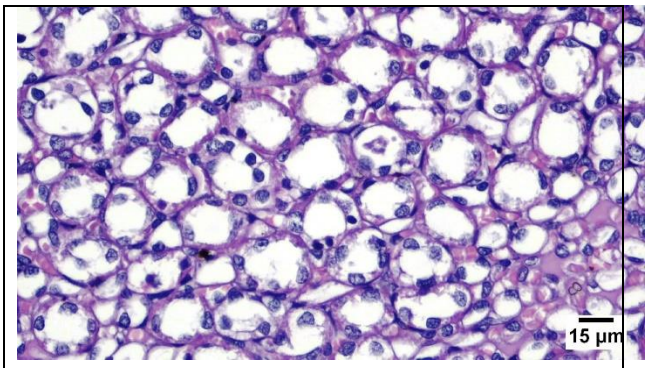
Pho. (2): Photomicrograph of kidney, PC group showing diffuse necrosis in the renal tubular epithelium with pyknotic nuclei and congested blood vessels (H&E).



Pho. (3): Photomicrograph of kidney, TTT 5% group showing vacuolar degeneration of tubular epithelium (H&E).



Pho. (4): Photomicrograph of kidney, TTT 10% group showing accumulation of renal casts in the tubular lumen (arrow) (H&E).



Pho. (5): Photomicrograph of kidney, TTT 15% group showing apparently normal renal medulla (H&E).

References

Aebi, H. (1984): Catalase in vitro. In: Methods of Enzymology; 105: 121–126.

Adriana E., Alina S., Vasile R., Cristina A., Corneliu A., And Sorana D. (2022): Protective Effect of Liposomal Epigallocatechin-Gallate in Experimental Gentamicin-Induced Hepatotoxicity. *Antioxidants*, *11*(2), 412

Agrarian, S. Hojjati, R. Ahrari, M. Bijad, E. Deris, F. Lorigooini Z. (2020): Ruta grave lens and rutin, as its major compound: investigating Their effect on spatial memory and passive avoidance memory in Rats Pharm. Biol., 58 (2020), pp. 447-453.

Ashtiani S., Jafari M., Najafi H. and Ahmadi M. (2013): Protective effects of Salvia Officinal is extract against gentamicin- induced nephrotoxicity in rat Journal of Kermanshah University of Medical Sciences. 2013; 17(4): 212-220.

Asrani, S.K; Devarbhavi, H; Eaton; J. and Klamath, P.S (2018): Burden of Liver diseases in the world. *J Hepatol*.70 (1):151-171.

Attalla, N., Mokhtar, F., Elekhnawy, E., Heneidy, S., Ahmed, E., Madeline, S., Negm, W., and El-Kareem, A. (2022): Mechanistic Insights on the In Vitro Antibacterial Activity and In Vivo Hepatoprotective Effects of Salvinia auriculata Aubl against Methotrexate-Induced Liver Injury. Pharmaceuticals (Basel), 15(5), 1-26.

Byrne, C. and Targher, G. (2022). Non-alcoholic fatty liver disease-related risk of cardiovascular disease and other cardiac complications. Diabetes Obese Metab, 24 Suppl 2, 28-43.

Camille F., Eleonore D., Alexandre B., Christine T., Arnaud B., MichaelM., Laurent M. and Christophe S. (2023): Dynamited ultra-low dilution of Ruta grave lens disrupts plasma membrane organization and decreases migration of melanoma cancer cell. Journal of Cell Adhesion &Migration, Volume 17.

Carlberg, I. and Mannervik, B. (1985): Glutathione Reductase. Methods in Enzymology, 113, 484-490.

Dana N., Hamed R. and Khalid M. (2020): Effect of introducing the Jordanian common rue (Ruta chalepensis L.) on blood lipid profile in Adult male Sprague Dawley rats toxified with Paracetamol. Journal of the Saudi Society for Food and Nutrition (JSSFN), 13(1), 17-

Ellefson R.D. and Caraway W.T., (1976): Fundamentals of clinical Chemistry. *Edition Tietz New*, p506.

Fas sati P. and Perncipe L., (1982): Serum Triglycerides Determined Calorimetrically with an Enzyme that Produces Hydrogen Peroxide. Clinical Chemistry, *Scientific Research Publishing*, 28, 2077-2080.

Fossati, P.; Principe, L. and Berti, G. (1980): Enzymatic colorimetric method of determination of urea in serum. Clin.Chem.6(18) 499-502.

Friedewald W.T., Levy R.I. and Fredrickson D.S., (1972): "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge," *Clinical Chemistry,* vol. 18, no. 6, pp. 499–502.

Gilbert S. and Weiner D. (2022): National Kidney Foundation Primer on Kidney Diseases, E-Book. Elsevier Health Sciences.

Gravot A., Larbat R., Hehn A., Lievre K., Gontier E., Goergen J., Bourgauda F. (2004). Cinnamic acid 4-hydroxylase mechanism based inactivation by psoralen derivatives: cloning and characterization of a C4H from a psoralen producing plant-Ruta grave lens-exhibiting low sensitivity to psoralen inactivation. Arch. Biochem. Biophys. 422: 71 80.

Henry, R. (1974): Creatinine measurement with colorimetric method. In clinical Chem., Principles and technics. Second edition, Haper And Row puplishers. hepatocytes. Cancer Lett, 97: 61-67.

Hashemi K., Sadeghpour H., Gholampour A., Mirzaei J. (2011). Survey the antifungal effect of root ethanolic extract of Ruta grave lens on Saprolegnia. Spp. Int. Con. Biotech. Environ. Manage., 18: 19-23.

Kannan R. and Babu U. (2012): Identity and pharmacognosy of Ruta graveolensLinn. Anc. Sci Life. 32 (1): 16-19.

Kathirvel P, Poonkodi K, Gomathi K, Deepadevi S. and Dhivya A. (2017): GC-MS analysis and in vitro antioxidant activities of ruta graveolens l. From western ghats region –south INDIA. Asian J Pharm Clin Res. 2017;10(5):297.

Kermanizadeh, A., Valli, J., Sanchez, K., Hutter, S., Pawlowska, A., Whyte, G., Moritz, W., and Stone, V. (2022): Particulate and drug Induced toxicity assessed in novel quadruple cell human primary hepatic disease models of statuses and pre-fibrotic NASH. Arch Toxically, 96(1), 287-303.

Khori V., Nayebpour M., Semnani S., Golalipour M., Marjani A. (2008): Prolongation of AV nodal refractoriness by Ruta grave lens in isolated rat hearts: Potential role as an antiarrhythmic agent. Saudi medical journal , 29:357-363.

Kuzovkina I., Al-terman I., Schneider B. (2004): Specific accumulation and revised structures of acridone alkaloid glucosides in the tips of transformed roots of Rutagraveolens. Photochemistry, 65: 1095- 1100.

Lopez-Virella M., Stone S., Ellis S. and Collwell G., (1977): Cholesterol determination in density lipoproteins separated by their different in Method; *Clinical Chemistry,* (5) 23: 882-886.

Mahmoud A., Ahmed R., Soliman H., Salah M. (2015): Ruta graveolens and its active constituent rutin protect against diethylnitrosamine induced nephrotoxicity through modulation of oxidative stress. J App Pharm Sci., 2015, 5: 16-21.

Makni M, Fetoui H, Gargouri N, Garoui EM, Jaber H, Makni J, Boudawara T, et al. (2008): Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in ω-3 and ω-6 fatty acids in hypercholesterolemia rats. Food and chemical toxicology 46: 3714 3720.

Malika H, Samira B, Zine K. (2017): Effect of Ruta chalepensison zinc, lipid profile and antioxidant levels in the blood and tissue of Streptozotocin induced diabetes in rats fed zinc-deficient diets. Can J Diabet 42 (4). DOI: 10.1016/j.jcjd.2017.08.239.

Mitra N., Mehrana J., Hanie A., and Mehdi N. (2019): Effects of Ruta graveolens total and flavonoids extracts on rat blood glucose, Cholesterol, triglycerides and urea comparing synthetic drugs. N U S A N T A R A B I O S C I E N C E. Vol. 11, No. 1, pp. 23-29.

Morrison, A., and Portilla D. (2019): Lipid peroxidation and the kidney Cellular Antioxidant Defense Mechanisms, CRC Press, pp. 173-184

Mohammad H., Dalia I., Heba M., Mohamed A., Nora M., Mai M., Abdulaziz M., and Hamad S. (2024): Preparation and in vivo evaluation of nano sized cubosomal dispersion loaded with Ruta graveolens extracts as a novel approach to reduce asthma-mediated lung inflammation. Saudi Pharmaceutical Journal Volume 32, Issue 3, 101968.

Nishikimi, M., Appaji, N. and Yagi, K. (1972): The occurrence ofsuperoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen.Biochem. Biophys.Res.Commun.;46(2): 849 854

Osama M., Mohamed b., and Maywood A. (2017): Assessment of the Preventive Effects of Salvia officinal is and Ruta grave lens Ethanol Leaf Extracts on Chlorpyrifos-and Methyl-induced Renal Toxicity and Oxidative Stress in Albino Rats. International Journal of Prevention and Treatment. (62):34-44.

Oun R., Moussa Y. and Wheate N. (2018): The side effects of platinum based chemotherapy drugs: a review for chemists. Dalton Trans., 47 (19) , pp. 6645-6653.

Pawankar, R. Hayashi, M. Yamanishi, S. Igarashi T. (2015): The paradigm of cytokine networks in allergic airway inflammation Curr. Opin. Allergy Clin. Immune. 15 (2015), pp. 41-48,

Park, H., Lee, C., Jung, I., Lee, J., Jeong, Y., Chang, J., Chun, S., Kim, M., Choi, I., An, S., Shin, Y., Yom, S., Park Y. (2009): Quercetin regulates Th1/Th2 balance in a murine model of asthma Int. Immunopharmacol. 9, pp. 261-267.

Paellas Viand Huber T. (2022): Kidneys control inter-organ homeostasis. Nat. Rev. Nephrol., 18 (4), pp. 207-208

Ranasinghe R., Mathai M. and Zulia A. (2023): Review article of Cytoprotective remedies for ameliorating nephrotoxicity induced by renal oxidative stress. Life Sciences , Volume 318, 121466

Reeves, P.; Nielsen, F. and Filmy, G. (1993): Purified diets for laboratory rodents: Final report of the American Institute of Nutrition writing committee on the reformulation of the AIN- 76 a rodent diet. J. **.Nurtr. 123(51): 1939 – 1951.**

Roy, E., (1970): Colorimetric determination of Co. St Louis. Toronto. Princeton; 1088-1273

Sahar Y, Eman M, and Nabil H. (2022): Effect of Ruta grave lens L. and Euphorbia peplus L. anti- inflammatory extracts on nutritional status of rats and the safety of their use. Indian Journal of Experimental Biology. 40(1):45-8.

Sales G. and Foresto R. (2020): Drug-induced nephrotoxicity.Rev. Assoc. Med. Bras., 66 , pp. s82-s90.

Sherwin, J. (1984): Liver Function. In Kaplan LA, Peace AJ,eds.Clinical chemistry ,theory,analysis,and correlation's Louis: Mosby 55(25):420 438.

Snedecor, G. and Cochran, W. (1980): Statistical methods.,7th Ed., Iowa State University Press, Ames, USA (90).

Toserkani A, Razi Jalali M, Najafzaheh H. (2012): Changes of lipid Profiles, glucose, and hemogram after administration of Ruta graveolens Extract in diabetic rats. Comp Clin Pathol 21: 1587-1592.

Young, D. (1990): Effect of drugs on clinical laboratory tests . Am. J. Clin. Pathol 3(7):6-12.

Zheng, W. V., Li, Y., Cheng, X., Xu, Y., Zhou, T., Li, D., Xiong, Y., Wang,S., & Chen, Z. (2022). Uridine alleviates carbon tetrachloride Induced liver fibrosis by regulating the activity of liver-related cells. J Cell Mol Med, 26(3), 840-854.

تأثير مستخلص زيت السداب على سمية الكبد والكلى المحدثة بالجنتامايسين على فئران التجارب

تسنيم صبحى فهمى & اسماء احمد محمد حسين

قسم التغذية وعلوم الأطعمة كلية الأقتصاد المنزلى جامعة حلوان

الملخص العربى

تهدف هذه الدراسة لمعرفة تأثير مستخلص زيت السداب على السمية الكبدية والكلوية المحدثة بالجنتاميسين على فئران التجارب. وقد تم استخدام 35 فأراً ألبينو من سلالة سبراغ داولي .وتم تقسيم الفئران بعد فترة التكيف إلى مجموعتين رئيسيتين (7 فار/ مجموعة)، وتم الاحتفاظ بالمجموعة الرئيسية الأولى على النظام الغذائي الأساسي كمجموعة ضابطة سالبة (-ve)، و تم حقن باقى الفئران (عدد= 28) تحت الجلد بالجنتاميسين (100 مل/كجم من وزن الجسم/ 7 أيام/العضو الذكري) لتحفيز السمية الكبدية والكلوية. بعد الحقن تم تقسيم الفئران إلى 4 مجموعات فرعية. كانت المجموعة الفرعية الأولى من الفئران بمثابة مجموعة ضابطة موجبة (+ve). أما المجموعات الفرعية الأخرى فقد غذيت على العلائق التجريبية التي تحتوي على مستويات مختلفة من مستخلص زيت السداب 5%، 10% و15% على التوالي. وفي نهاية الفترة التجريبية (6 أسابيع) تم قياس وظائف الكبد والكلى. كما تم قياس الانزيمات المضادة للأكسدة. وتم إجراء الفحص النسيجي ا للكبد. وأشارت النتائج إلى انخفاض إنزيمات الكبد ALT وAST وALP في المجموعات 3،4 و5 على التوالي مقارنة مع مجموعة الضابطة الموجبة . ولاحظ تحسن فى مستويات حمض اليوريك والكرياتينين بشكل ملحوظ في جميع المجموعات التي تم اختبارها مقارنة مع مجموعة الضابطة الموجبة. علاوة على ذلك، تحسنت إنزيمات مضادات الأكسدة CAT، SOD، GSH في جميع المجموعات المعالجة مقارنة مع مجموعة الضابطة الموجبة . ونستنتج من الدراسة أن زيت السداب كان له تأثير جيد ضد تلف الكبد والكلى، لذلك توصي هذه الدراسة ان زيت السداب يمكن أن يكون مفيدًا للمرضى الذين يعانون من تلف الكبد والكلى

الكلمات المفتاحية: زيت السداب ، إنزيمات الكبد، تلف الكبد، الإنزيمات المضادة للأكسدة.