NON-NEURONAL PATHOLOGY OF ROTENONE IN SPRAGUE DAWLEY RATS: POTENTIAL PROTECTIVE EFFECT OF LITHIUM CHLORIDE

Running title: LiCl alleviates ROT-induced non-neuronal pathology in rats

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ABSTRACT

Rotenone (ROT), a mitochondrial complex I inhibitor, has been linked to Parkinson's disease (PD) since Betarbet and her colleagues reproduced the most features of the disease in a murine model challenged with ROT in 2000. Since that time, ROT is extensively used to model PD and there are no detailed reports investigating its adverse effects on non-neuronal tissues. Thus, the goal of our current study was to investigate the non-neuronal pathophysiology of ROT in rats. Additionally, the possible preventive effect of lithium chloride (LiCl) against pathology induced by ROT was examined. Forty male Sprague Dawley (SD) rats were divided into four groups: those treated with vehicle, ROT, ROT plus LiCl, and LiCl. ROT (2 mg/kg b.w.) and LiCl (60 mg/kg b.w.) were respectively administered subcutaneously and orally, five times a week for 5 weeks. At the end of each treatment, animals' body weight and gastrointestinal functions were assessed. Blood samples and tissue specimens from liver, kidney, stomach, adrenal gland and testis were obtained, and adopted for biochemical and hsitopathological analysis, respectively. ROT resulted in gastrointestinal dysfunction, decreased the activity of some antioxidant enzymes and increased MDA levels. Histopathologically, ROT characteristically injured blood vasculature in different body organs and consequently damaged the parenchyma of these organs. Co-treatment of rats with ROT and LiCl alleviated most adverse effects that produced by ROT. In conclusion, ROT adversely affects non-neuronal organs in rats, most notably liver, kidney, stomach, adrenal gland and testis. Against ROT, LiCl has the potential to provide observable protection against ROT-induced damage in these organs.

Keywords: Rotenone, lithium chloride, non-neuronal, rats, adrenal

INTRODUCTION

ROT is a naturally occurring alkaloid derived from the roots, seeds and stems of a number of tropical plants such as Tephrosia, Derris, Lonchocarpus and Mundulea species (Ahmadi *et al.,* 2003). It was discovered in South America and Southeast Asia hundreds of years ago. In that time, Peruvian natives used crude extracts of ROT-containing plants to catch fish (Islam, 2006). ROT was first isolated by the Japanese Chemist Nagai Nagayoshi who isolated a pure crystalline compound from Derris elliptica and called it ROT around 1900 (La Forge *et al.,* 1933). ROT

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had been extensively used since 1932 as a piscicide for controlling naissance fish in North America and nowadays, it is recognized as the most environmentally benign pesticide and piscicide worldwide (Ma et al., 2018).

ROT is classified by World Health Organization (WHO) as a moderately hazardous agent (a class II pesticide) and Environmental Protection Agency (EPA) has prohibited all food uses of ROT since 2006 and only allowed its registration for piscicidal uses with strict regulations (Ma et al., 2018). As ROT is highly hydrophobic, it is able to easily cross the cell membranes without need to specific transport receptors. Inside cells, ROT strongly inhibits mitochondrial complex I resulting in disruption of adenosine triphosphate (ATP) synthesis and reactive oxygen species (ROS) production (Degli Esposti, 1998).

Recently, ROT has been implicated as a potential risk factor for PD since it has shown to reproduce most features of PD in murine models (Betarbet et al., 2000) and increasing the evidence that demonstrates alterations in complex I in PD patients (Tanner et al., 2011). Additionally, the importance of ROT as a risk factor for Parkinson's disease was strengthened by the dopamine neurons' innate susceptibility to changes in mitochondrial activity and oxidative stress (Dhillon et al., 2008). Therefore, linking of ROT exposure to increasing risk of PD gives much attention to it as a neurotoxin and incites researchers to heavily studying its effects on both central and peripheral nervous system. To our knowledge, there are no in vivo detailed reports describing non-neuronal pathology of ROT.

LiCl is a famous pharmacological treatment for mood disorders (Skold et al., 2021) and recently, it has been suggested to have neuroprotective effects both in in vitro and in vivo studies (Fan et al., 2015). For instance, Chen et al. (2004) demonstrated that lithium protected SH-SY5Y cells against apoptotic cell death produced by 6-OHDA. Wei et al. (2001) showed that lithium could protect against brain damage induced by focal cerebral ischemia in rats. Ala et al. (2021) showed that LiCl protected against optic nerve injury in rats through anti-apoptotic mechanism.

Taken all together, our current study was designed to investigate non-neuronal pathology as the result of ROT treatment in rats and the potential protective role of LiCl against ROT-induced pathological alterations.

MATERIALS AND METHODS

Animals and chemicals
Forty male SD rats (8 weeks old and 200 - 250 g weight) were obtained from Animal House, Department of Pathology, Faculty of Veterinary Medicine, Assiut University. Rats were housed 5 per a cage and maintained under standard laboratory conditions (12 h light/dark cycle, 22±2 °C and 60% humidity). All rats had free access to food and water during the experiment. ROT (CAS No. 83-79-4) and LiCl (CAS No. 7447-41-8) were purchased from Sigma-Aldrich (Germany).

Experimental design
All experimental procedures were done according to the European Union Council guidelines (86/60/EU) and approved by the animal ethics committee, Faculty of Veterinary Medicine, Assiut University (approval number 06/2024/0224).

After acclimatization for one week, rats were randomly assigned to 4 groups (10 rats each). Vehicle-treated group received 50 µl dimethyl sulfoxide (DMSO) + 950 µl sunflower oil/kg b.w. by subcutaneous route and acts as a control for ROT-treated animals. ROT-treated group received 2 mg ROT dissolved in 50 µl DMSO + 950 µl sunflower oil/kg b.w. by subcutaneous

route. ROT and LiCl group co-administered 2 mg ROT dissolved in 50 μl DMSO + 950 μl sunflower oil/kg b.w. by subcutaneous route and 60 mg LiCl/kg b.w. by oral route. LiCl-treated group received 60 mg LiCl/kg b.w. by oral route. Rats were treated 5 days a week for 5 weeks.

Body weight and general health
Body weight was taken for each rat at the beginning and the end of treatment periods. Weight gain or loss was assessed according to the equation [(start body weight – end body weight)/start body weight × 100]. Animals were also observed daily to check for bradykinesia (absent rearing and/or slowed movement), rigidity (hunched posture and increased tail tone), dystonia (clenched paws) and signs of dehydration (tenting skin or dry mucus membranes).

Evaluation of GI dysfunction
One hour stool collection test
One day before euthanization of animals between 8 - 11 am, each rat was put in a clean plastic cage with no food and water. Stools were then collected immediately after expulsion for one hour and put in a sealed tube. The stools in the tube was weighed to give a wet weight and then dried over night at 65 °C to give a dry weight. Water contents was calculated according to the equation [(wet weight – dry weight/wet weight) × 100]. The three values were normalized to body weight (Li *et al.,* 2006; Greene *et al.,* 2009).

Solid gastric emptying test
It was used to assess whether rats developed gastroparesis at the end of the experiment (Johnson *et al.,* 2018). After fasting for an overnight, rats were humanely killed and the stomachs were weighed before and after flushing with distilled water to determine the weight of the stomach contents, an indicator of the gastric emptying capacity. All values were normalized to rats' body weight.

Blood and serum samples
Before sacrificing animals, a blood sample was collected from the medial canthus of the eyes of each rat and divided into two parts. The first part was collected into a clean blood tube containing anticoagulant (EDTA) for determination of red and white blood indices using Exigo Veterinary Hematology Analyzer (Sweden), Department of Pathology, Faculty of Veterinary Medicine, Assiut University. The second part was collected into a blood tube without anticoagulant for serum preparation.

Biochemical analysis
Prepared serum was used to measure the activities of CAT and SOD, and the levels of GSH and MDA. CAT activity was determined based on its ability to breakdown the hydrogen peroxide (H_2O_2) according to the method of Aebi (1984). The activity of SOD was determined based on its ability to inhibit the autooxidation of epinephrine at an alkaline medium according to the method of Misra & Fridovich (1972). GSH levels were measured according to the method of Ellman (1959). Levels of MDA as a lipid peroxidation marker were assessed according to the method of Ohkawa *et al.* (1979). Total protein concentration was measured based on the method of Lowry *et al.* (1951).

Histopathological examination
At the end of the experiment, specimens from liver, kidneys, stomach, adrenal glands, ileum, colon and testes were obtained, washed with normal saline and fixed in 10% neutral buffered formalin for 24 - 48 h. They were then dehydrated in a graded alcohol series, cleared in xylene and embedded in paraffin wax, cut at 4 μm thickness and stained with hematoxylin and eosin (H&E) **(Bancroft & Stevens, 1990)**. Stained tissue sections were examined under light microscopy (Olympus CX31, Japan) and photographed

using a digital camera (Olympus, Camedia C-5060, Japan).

Histopathological scoring using incidence method

Three photomicrographs for each characteristic lesion were taken from three different specimens from the liver, kidney, stomach, adrenal gland and testis in different treated groups. The incidence of the lesions in different treated groups was calculated (Gibson-Corley *et al.,* 2013).

Statistics

Data were expressed as mean±SEM and statistically analyzed by one-way ANOVA followed by Tukey post-hoc test for multiple comparisons. A SPSS program (version 16.0) was used for analysis. P≤0.5 was considered significant.

RESULTS

Clinical signs

There were no deaths during the experiment and the body weight of experimental animals was not significantly changed between different groups (Figure 1A). Compared to vehicle-treated rats, ROT treatment resulted in some systemic illnesses including loss of rearing, slow movement, hunched posture and piloreaction. These clinical signs markedly decreased in ROT and LiCl-treated group, and not found in LiCl-treated rats (data not shown).

One hour stool analysis

Water content of collected stool during an hour (P=0.969) was markedly decreased by ROT treatment compared to vehicle-treated rats. On the other hand, concomitant treatment of rats with ROT and LiCl increased water content (P=0.986), compared to ROT-treated rats. Treatment of rats with only LiCl did not affect the water content of the collected stool (Figure 1B).
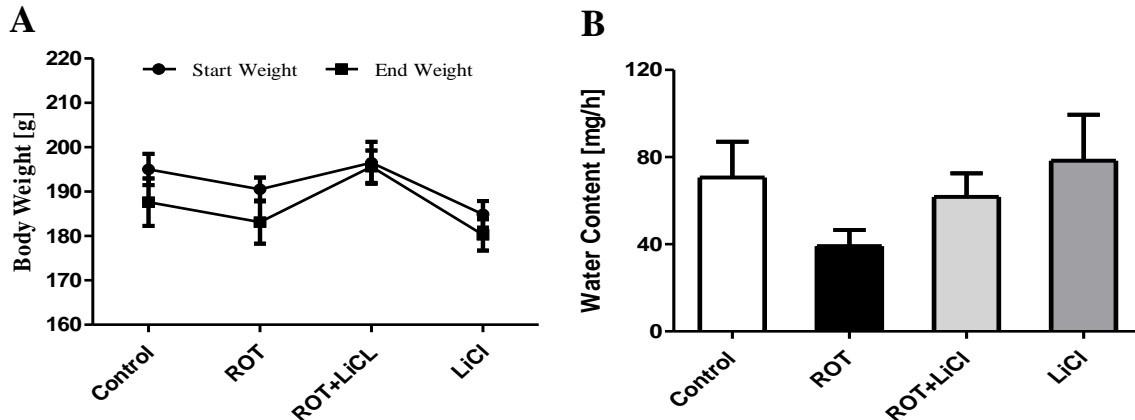

Figure 1: A) Body weight of rats at the beginning and the end of the experiment. There are no differences between different treated groups. **B**) Effect of co-treatment with ROT and LiCl on the water content in rats' stools. Co-administration of ROT and LiCl markedly increases water content compared to ROT-treated group. LiCl alone does not affect water content.

Solid gastric emptying

Treatment of rats with ROT significantly increased the weight of filled (P=0.031) and empty (P=0.05) stomachs, and the stomach content (P=0.048) compared to rats received vehicle. In ROT and LiCl group, the weight of filled (P=0.086)) and empty (P=0.112) stomachs, and stomach content (P=0.098)) was markedly decreased when compared to ROT-treated rats. Rats in LiCl-treated group did not differ from those treated with vehicle (Figure 2).
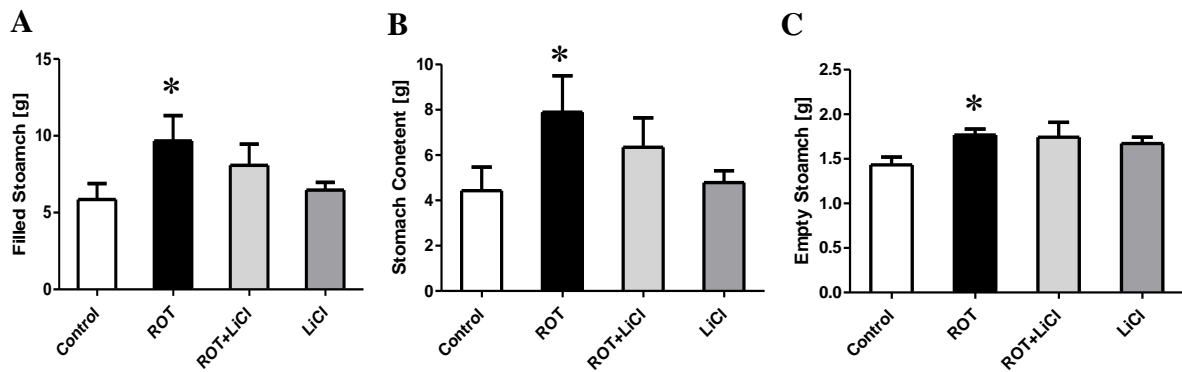
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A

B

C

Figure 2: Effect of ROT and LiCl treatment on the weight of filled **(A)** and empty **(B)** stomachs, and stomach content **(C)** in rats. ROT significantly increases the all three parameters compared to vehicle-treated group. Co-treatment of rats with ROT and LiCl markedly decreases the all three parameters compared to ROT-treated rats. LiCl alone does not affect the three parameters.

Biochemical findings

ROT treatment resulted in slight increase in CAT (P=0.69) and significant decrease in SOD (P=0.001) activities, significant decrease in the GSH concentration (P=0.045)) and significant increase in MDA level (P=0.001) compared to vehicle-treated animals. Concomitant treatment of rats with ROT and LiCl led to a marked increase in the activity of CAT (0.17), did not affect the activity of SOD (P=1.00), significant increase in GSH concentration (P=0.05) and significant decrease in MDA level (P=0.005) compared to ROT-treated group. Treatment of animals with LiCl alone did not significantly affect all parameters (Figure 3).
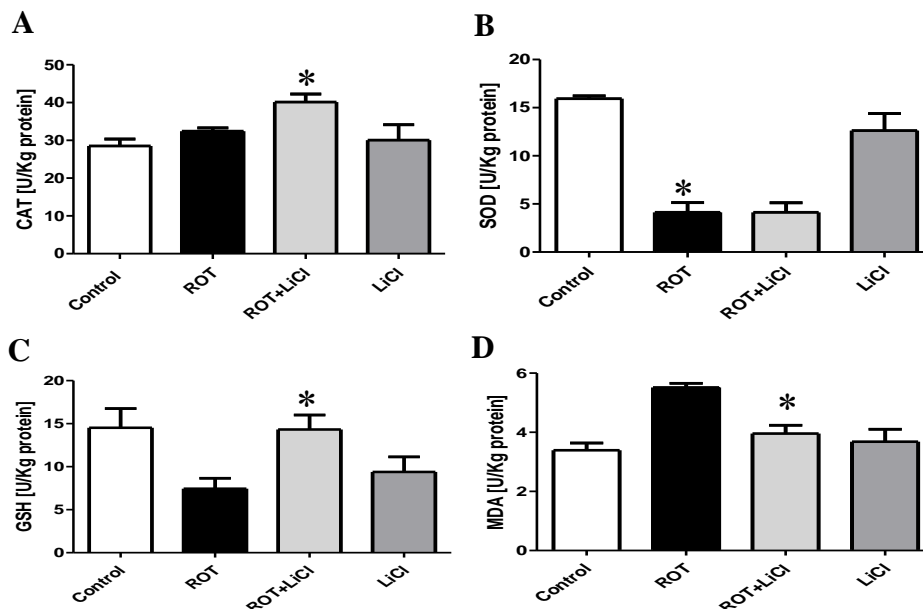
A

B

C

D

Figure 3: Effect of ROT and LiCl on the activity of CAT and SOD, and levels of GSH and MDA in rats' serum. **A)** Co-treatment of rats with ROT and LiCl markedly increases CAT activity compared to the slight increase by ROT alone. **B)** ROT significantly decreases SOD activity, and co-treatment with ROT and LiCl does not affect the activity of the enzyme. **C)** Co-treatment of rats with ROT and LiCl significantly alleviates the decrease in GSH produced by ROT. **D)** Co-treatment of rats with ROT and LiCl significantly decreases formation of MDA compared to ROT-treated group. LiCl alone does not affect the activities both CAT and SOD, and MDA level. It insignificantly decreases the GSH concentration.

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Histopathology

Microscopic examination of H&E-stained liver sections from vehicle-treated rats showed hepatocytes with well-preserved cytoplasm and well-defined centrally located vesicular nuclei (Figure 4A). Treatment of rats with ROT resulted in dilatation and congestion of blood vessels including central and portal veins, and blood sinusoids (Figure 4B,C) and endothelial damage and thrombosis of some blood vessels (Figure 4B,D). There was also activation of Kupffer cells and vacuolar degeneration of hepatocytes (Figure 4B), and focal areas of hepatic necrosis with mononuclear cellular infiltration (Figure 4 E, F). Kidney sections from vehicle-treated rats showed normally arranged glomeruli and renal tubules with normal histological appearance (Figure 5A). ROT treatment consistently caused glomerular congestion (Figure 5B), swelling (Figure 5C) and sclerosis (Figure 5D). ROT also produced hemorrhages (Figure 5D), and swelling and necrosis of renal tubular epithelium in some renal tubules (Figure 5B). Examination of the fundus of the stomach showed normal gastric glands with cuboidal cell lining in vehicle-treated group (Figure 6A). In ROT-treated rats, there were slight damage (Figure 6B) and dilatation of the gastric glands (Figure 6C). Some cases exhibited varying degree of gastric damage with mononuclear cell infiltration and hypertrophy of muscularis mucosa (Figure 6D). In vehicle-treated group, adrenal gland appears consisting of the outer capsule, the cortex (zona glomerulosa, zona fasciculate and zona reticularis) and the medulla (Figure 7A). Treatment of rats with ROT caused clear vacuolation of zona fasciculata cells (Figure 7B) and severe congestion of medullary blood vessels (Figure 7C). Testis in vehicle treated rats showed normally arranged seminiferous tubules with complete seminiferous tubular epithelium and many sperms in their lumina (Figure 8A). ROT treatment induced damage to the seminiferous tubular epithelium (Figure 8B), and loss of sperms (Figure 8C) and cellular aggregations (Figure 8D) in some tubules. Co-administration of rats with ROT and LiCl markedly decreased the intensity of pathological lesions in the liver (Figure 4G), kidney (Figure 5E), stomach (Figure 6E), adrenal gland (Figure 7D,E) and testis (Figure 8E). No apparent changes could be seen in LiCl-treated rats (Figures 5 – 8).

Incidence of pathological lesions

The incidence of the lesions as the result of ROT treatment was significantly higher than in vehicle treated group. On the other hand, co-treatment with ROT and LiCl significantly reduced the incidence of these pathological lesions compared to ROT-treated group. Nearly, there was no difference between LiCl- and vehicle-treated groups regarding the pathological lesions (Figure 9).
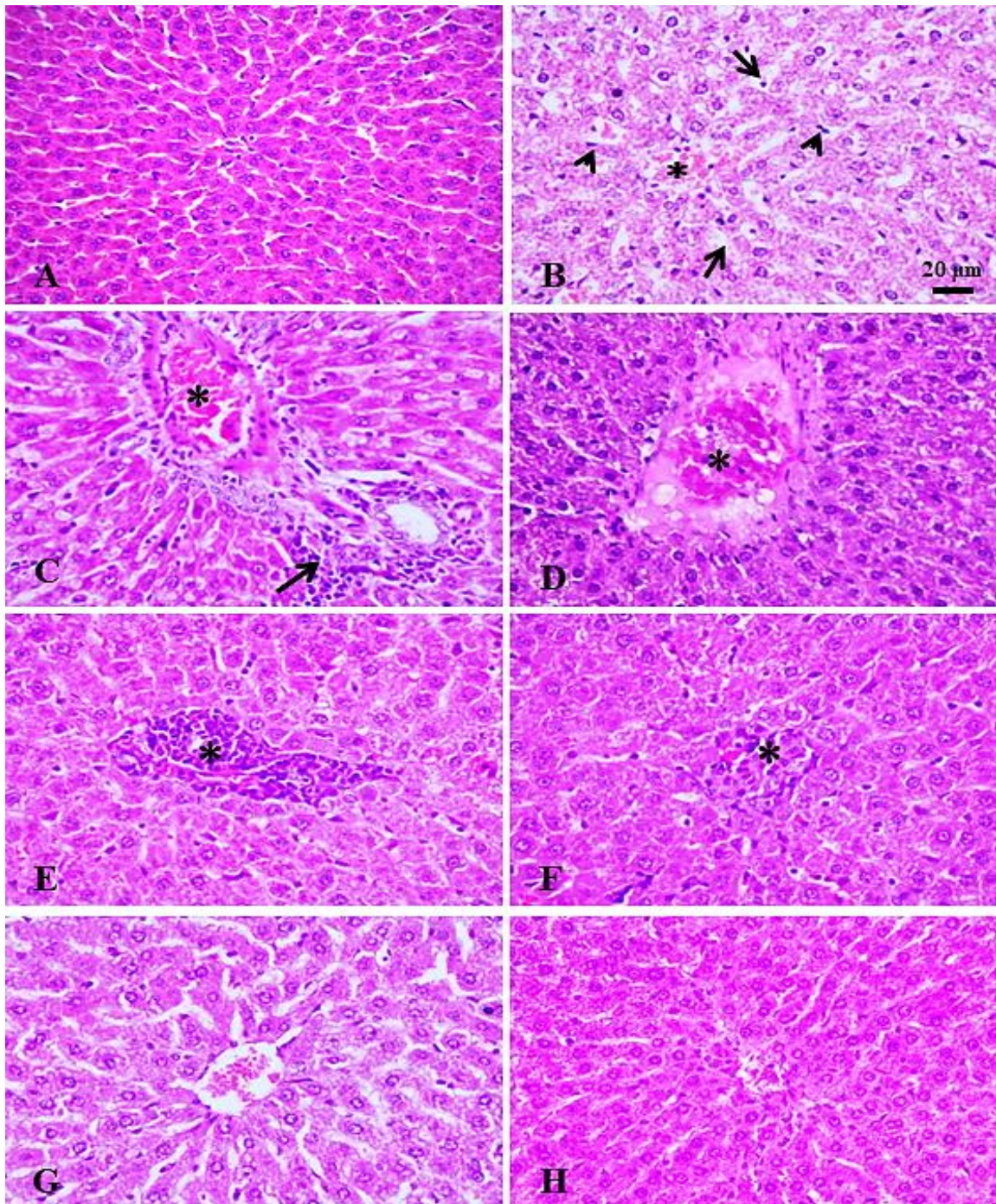
Figure 4: Representative photomicrographs for the liver in different treated groups. **A)** A vehicle-treated rat shows normal hepatic architecture. Hepatocytes appear with well-preserved cytoplasm and well-defined centrally located vesicular nuclei. **B)** A ROT-treated rat shows congestion and endothelial damage in central veins (asterisk), dilatation of blood sinusoids (arrows) and Kupffer cell activation (arrow heads). **C)** A ROT-treated rat shows dilatation and congestion of portal vein (asterisk), and periportal lymphocytic aggregation (arrow). **D)** A ROT-treated rat shows thrombus formation (asterisk). **E)** A ROT-treated rat shows focal mononuclear cell aggregation (asterisk). **F)** A ROT-treated rat shows focal areas of hepatic necrosis with mononuclear cellular infiltration (asterisk). **G)** Co-administration of rats with ROT and LiCl alleviates most ROT-induced pathological lesions. **H)** There are no apparent lesions in LiCl-treated rats. H&E.
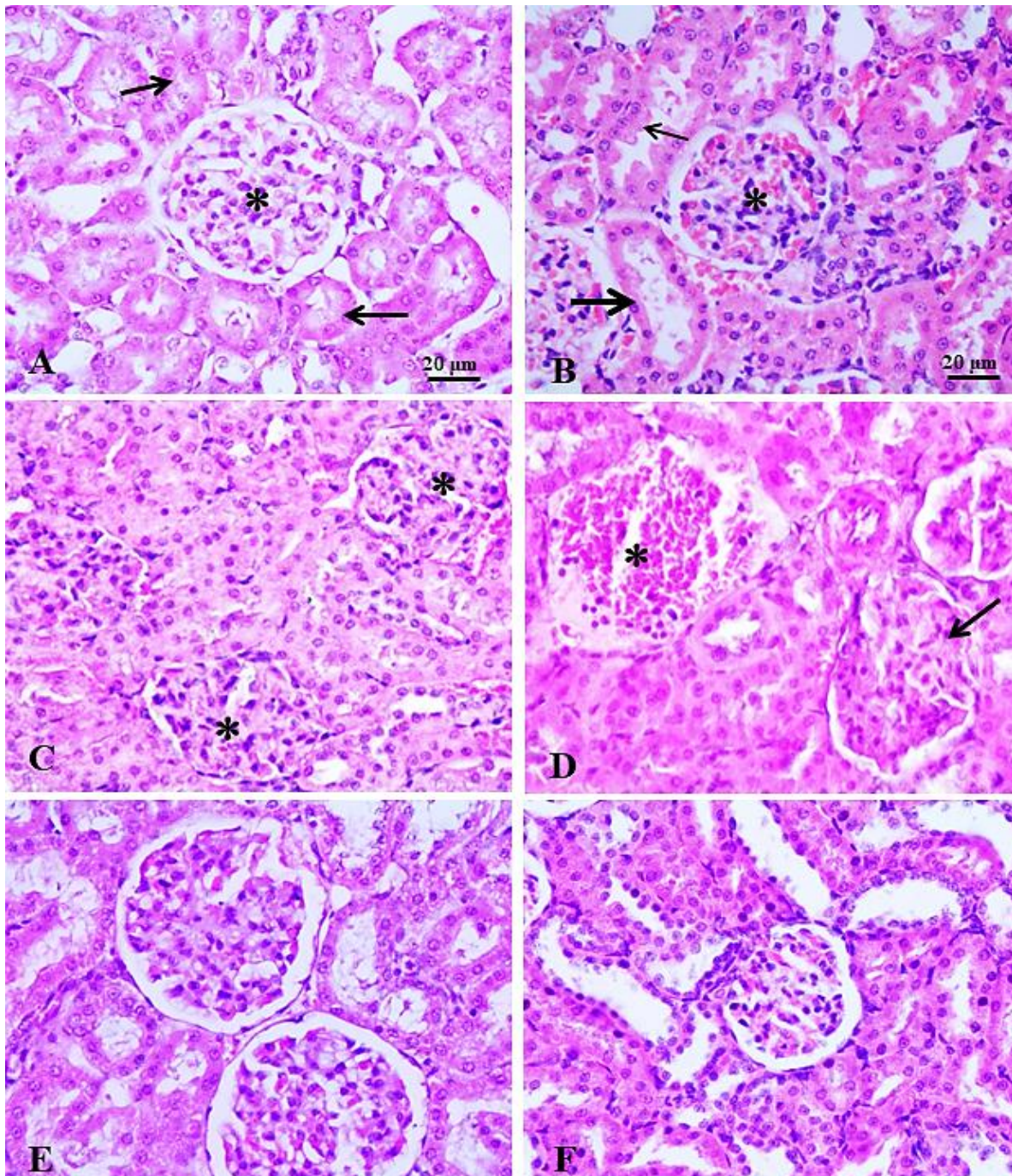
Figure 5: Representative photomicrographs for the kidney in different treated groups. **A)** A vehicle-treated rat shows normal histological structure of the kidney. Glomerulus (asterisk) and renal tubules (arrows). **B)** A ROT-treated rat shows glomerular congestion (asterisk), and swelling (thin arrow) and necrosis (thick arrow) of renal tubular cells in some tubules. **C)** A ROT-treated rat shows glomerular swelling (asterisks). **C)** A ROT-treated rat shows hemorrhages (asterisk) and glomerular sclersosis (arrow). **E)** Co-administration of rats with ROT and LiCl alleviates most ROT-induced pathological lesions. **F)** There are no apparent lesions in LiCl-treated rats. H&E.
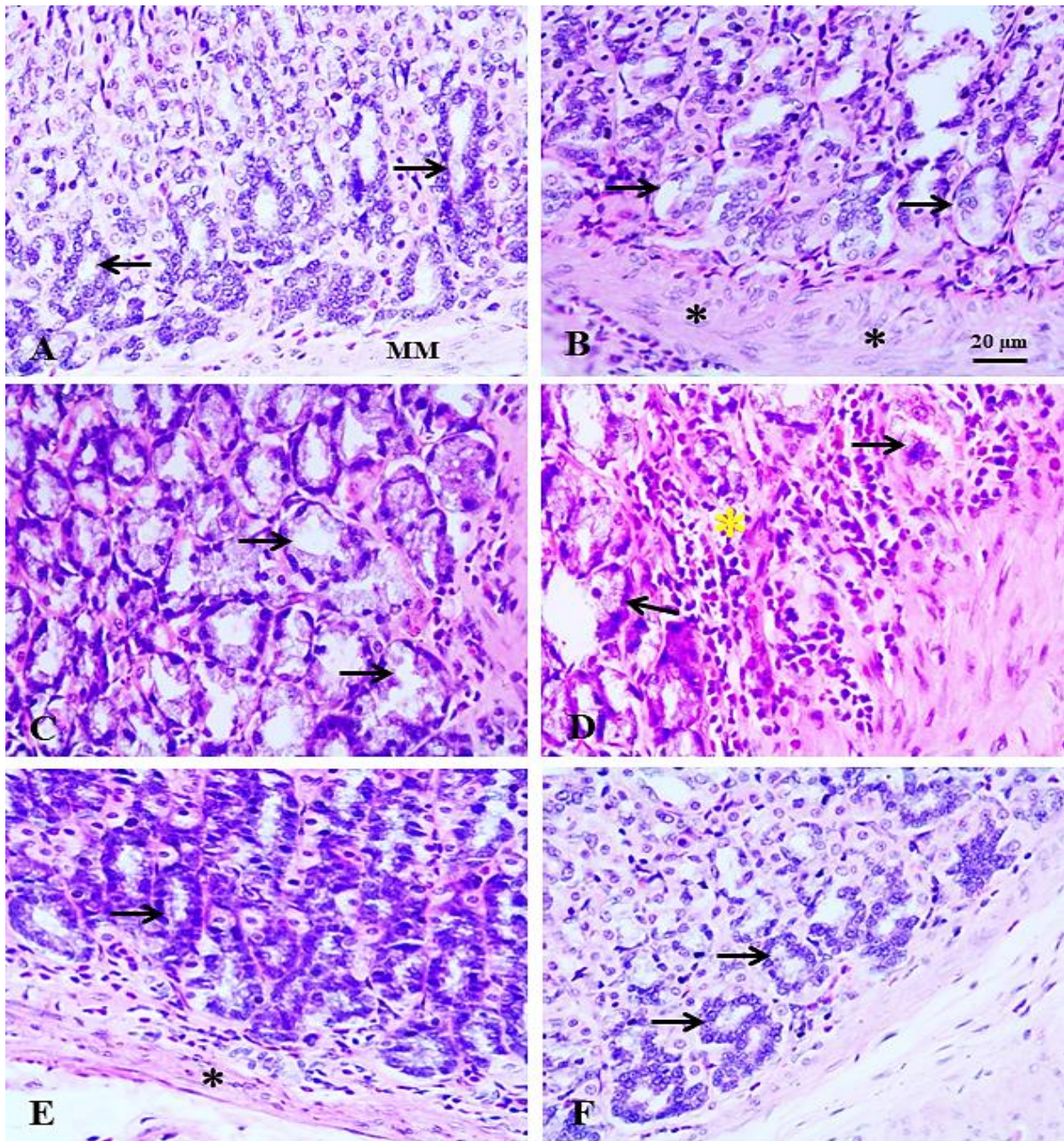
Figure 6: Representative photomicrographs for the stomach in different treated groups. **A)** A vehicle-treated rat shows normal histological appearance of the fundus region. Gastric glands (arrows) and muscularis mucosa (MM). **B)** A ROT-treated rat shows slight damage to the gastric glands (arrows) and mild hypertrophy of muscularis mucosa (asterisks). **C)** A ROT-treated rat shows dilatation of gastric glands with glandular secretion (arrows). **D)** A ROT-treated rat shows varying degrees of damage to the gastric glands (arrows) with mononuclear cell infiltration (asterisk). **E)** A rat co-treated with ROT and LiCl shows normally distributed gastric glands (arrows) with slight enlargement of muscularis mucosa (asterisk). **F)** A LiCl-treated rat shows normally distributed gastric glands (arrows).
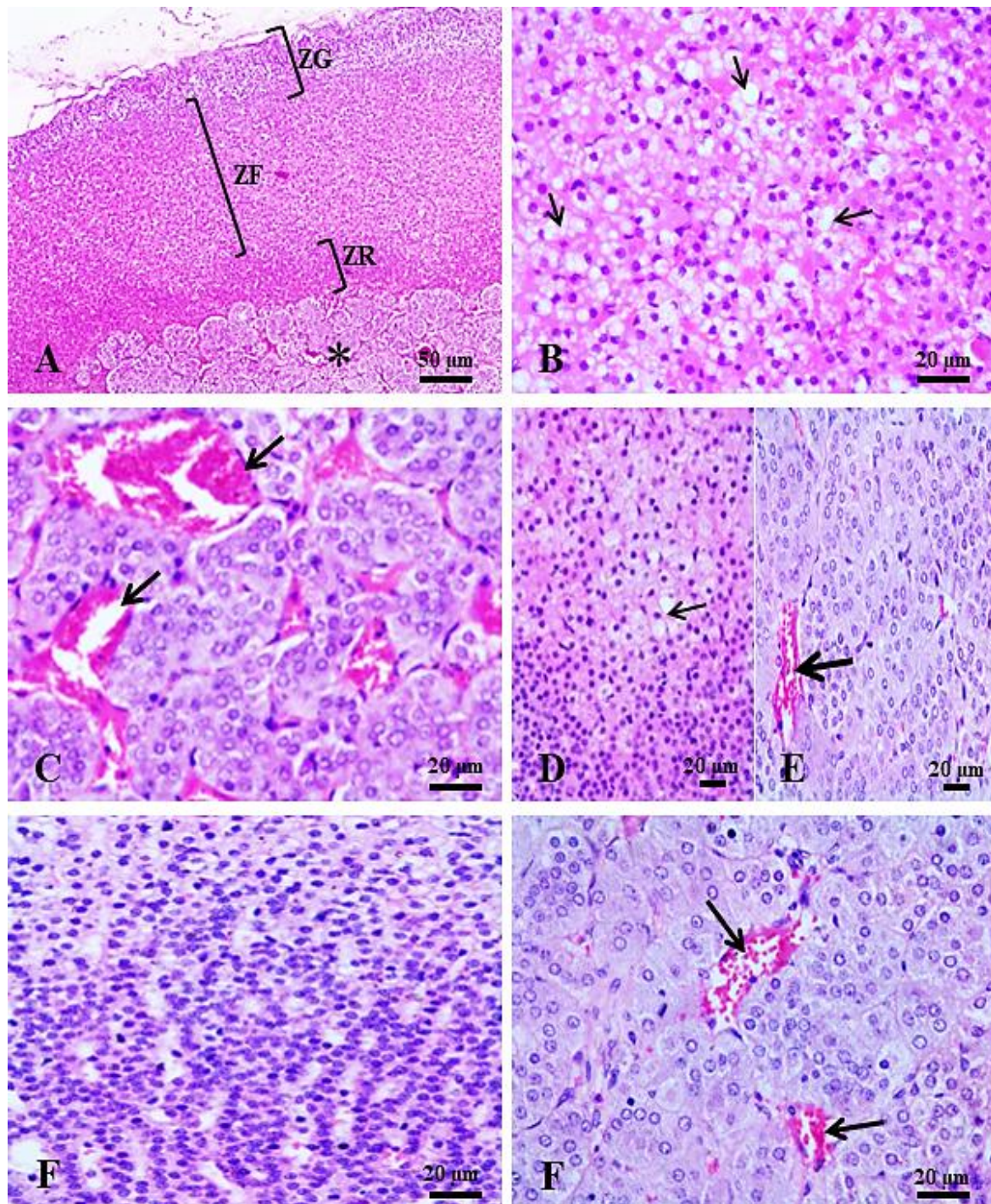
Figure 7: Representative photomicrographs for the adrenal glands in different treated groups. **A)** A vehicle-treated rat shows normal histological architecture of the adrenal gland. Zona glomerulosa (ZG), Zona fascicularis (ZF) and Z reticularis (ZR). **B)** A Rot-treated rat shows vacuolation of ZF cells (arrows). **C)** A ROT-treated rat shows severe congestion of medullary blood vessels (arrows). **D)** A rat co-treated with ROT and LiCl shows mild vacuolation of ZF cells (thin arrow) and congestion of medullary blood vessels (thick arrow). **E)** A LiCl-treated rat shows normal ZF cells. **F)** A LiCl-treated rat shows mild congestion of medullary blood vessels (arrow). H&E.
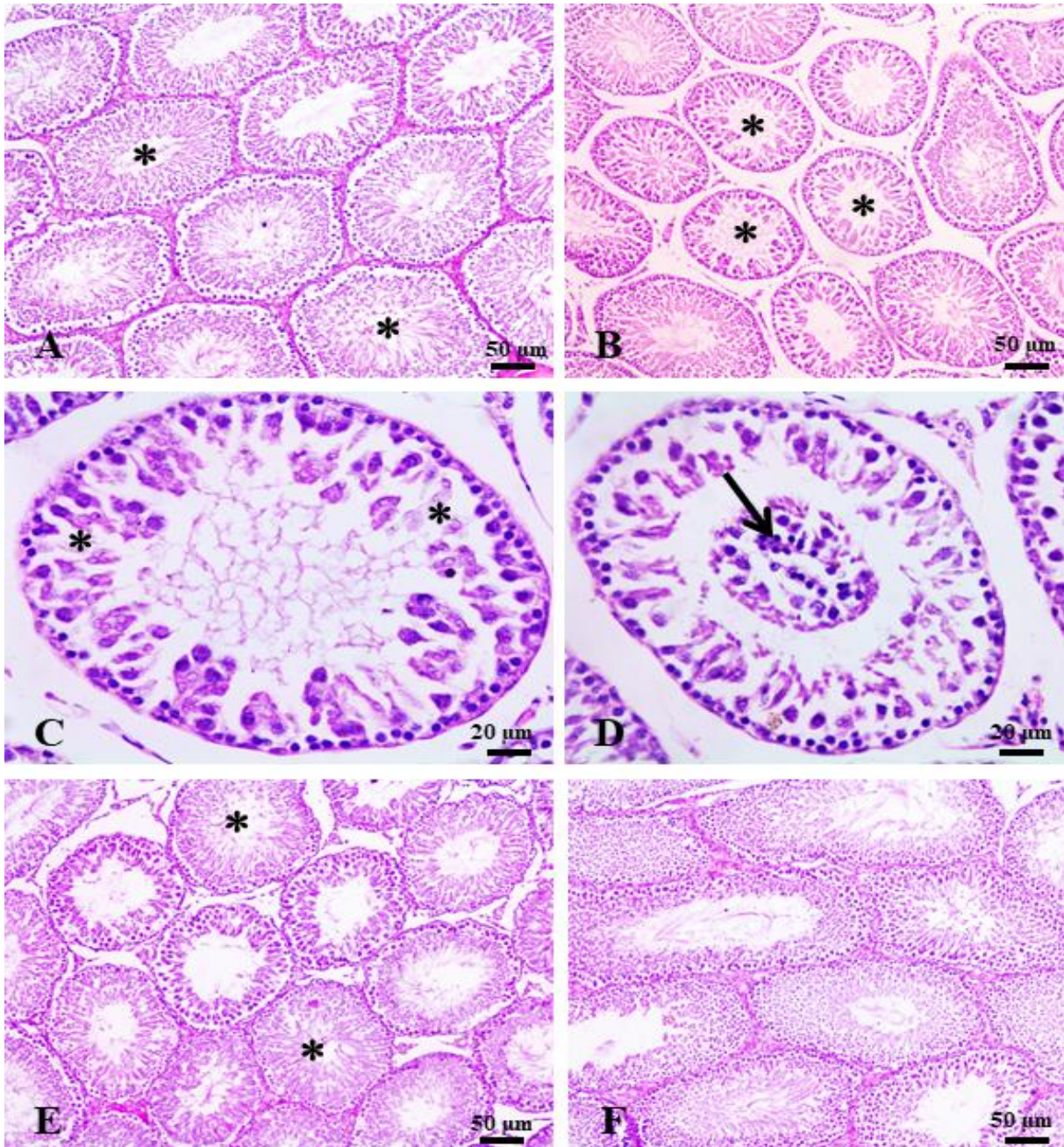
Figure 8: Representative photomicrographs for the testes in different groups. **A)** A vehicle-treated rat shows normal seminiferous tubular epithelial lining with presence of many sperms in the lumen (asterisks). **B)** A ROT-treated rat shows damaged tubular epithelium and loss of sperms (asterisks) in some seminiferous tubules. **C and D)** Higher magnification for a ROT-treated rat shows loss of tubular epithelial lining (asterisks) and cellular aggregations in the lumen (arrow). **E)** A rat co-treated with ROT and LiCl shows more or less normal seminiferous tubules with intact epithelial lining and sperms in the lumina (asterisks) compared to ROT-treated rats. **F)** A LiCl-treated rat shows seminiferous tubules similar, to a greater extent, to vehicle-treated rats. H&E.
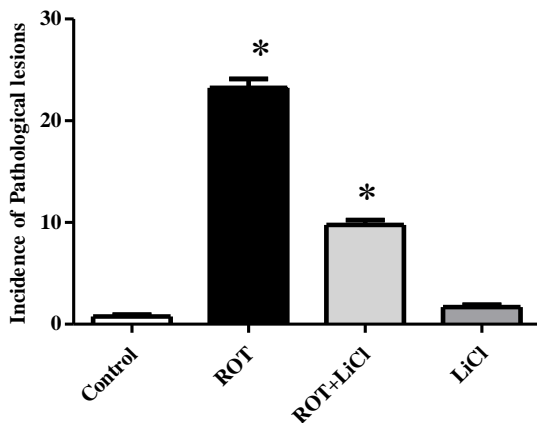
Figure 9: Incidence of the pathological lesions in different treated groups. ROT treatment significantly increases the incidence of the lesions compared to vehicle- treated group. Co-treatment with ROT and LiCl significantly reduces the incidence of these pathological lesions compared to ROT-treated group. There is no marked differences between LiCl- and vehicle-treated groups.

DISCUSSION

ROT, a mitochondrial complex I inhibitor, is usually used experimentally to model PD as it reproduced most of its behavioral, biochemical and hsitoapthological features according to Betarbet and her colleagues (2000). In our current report, we tried to investigate the non-neuronal pathology of ROT in rats which has not been unraveled well in the literature. Treatment of rats with ROT clinically led to some systemic illnesses such as loss of rearing and slow movement. These signs were specifically reported in ROT-induced murine models as the result of dopaminergic system deficiency (Miyazaki *et al.,* 2020). However Lapointe *et al.* (2004) considered these signs as a part of general health problems rather than a specific motor deficit.

ROT was also found to affect gastrointestinal functions, the non-motor sign that early complained by PD patients (Johnson *et al.,* 2018). It markedly decreased the water content and significantly increased the stomach residual content compared to vehicle-treated rats. Similar results were reported by Greene *et al.* (2009) and Drolet *et al.* (2009). Decreasing stool water content and increasing stomach residual content indicate the adverse effects of ROT on colon motility and gastric function, respectively. These effects of ROT on gastrointestinal function seem to be complex and were reported to be mediated by the action of ROT on central and enteric nervous system (Greene *et al.,* 2009). On the other hand, co-treatment of rats with ROT and LiCl caused marked but insignificant increase in stool water content and decrease in gastric residues compared to ROT-treated group. Insignificant results regarding gastrointestinal functions in our study might return to the variability in animals' response to ROT administration (Sherer *et al.,* 2003).

ROT was reported to increase oxidative injury, and decrease the efficiency of endogenous antioxidant defense mechanisms and the activity of antioxidant enzymes both in *in vitro* and *in vivo* studies (Alabi *et al.,* 2019: Kumar *et al.,* 2023). Likewise, treatment of rats in our study with ROT decreased the activities of CAT and SOD, deplete GSH concentration and raise MDA levels in the serum. These results show that ROT has systemic consequences since they correspond with damage to several body organs. In contrast to ROT, LiCl markedly raised GSH levels and decreased MDA levels in serum while slightly increasing CAT activity and having no effect on SOD activity. According to certain research, LiCl additionally contributed to ameliorate some of the alterations in oxidative stress parameters including GSH, MDA and nitric oxide (Tharwat *et al.,* 2023).

To our knowledge, there have been no reports describing observable histopathological alterations in non-neuronal tissues as the result of ROT treatment in animal models. Our current study revealed that ROT was found to damage rats' liver in the form of vacuolar degeneration and focal necrosis of hepatocytes, activation of Kupffer cells and mononuclear cell infiltration. Besides oxidative stress, injuries to blood vessels including endothelial damage and thrombosis might mediate different hepatic lesions induced by ROT. In parallel, some of these vascular changes as the result of ROT treatment were reported by Allen *et al.* (2009) and Radad *et al.* (2013). Additionally, Akinmoladun *et al.* (2018) and Wang *et al.* (2022) observed that ROT induced alterations in the levels of alanine amoinotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenease (LDH) and bilirubin, the indices that indicate hepatic damage. In the kidney, some ROT-treated rats showed congestion, swelling and sclerosis of glomeruli, kidney hemorrhages, and swelling and necrosis of renal tubular epithelium in some renal tubules.

Consistent to our findings, Jiang *et al.* (2017) found that ROT administration to rats damaged renal tissues. The authors also attributed those renal damages to oxidative stresses as evaluated by increasing formation of ROS and MDA, and decreasing the activities of some antioxidant enzymes such as SOD and GSH-Px (Jiang *et al.,* 2017). Gastric lesions due to ROT were mild and confined to the fundus, and seen in few numbers of ROT-treated animals. They were in the form of mild damage and dilatation of gastric glands, and hypertrophy of muscularis mucosa. These changes are, in part, explained by gastrointestinal dysfunction according to the results of one hour stool analysis and solid gastric emptying tests which are also coincide with the findings of Johnson *et al.*

(2018). ROT was also seen to disrupt the histology of adrenal gland; it resulted in clear vacuolation of ZF cells and congestion of medullary blood vessels. Likewise, Hassan *et al.* (2023) reported that intraperitoneal administration of ROT to rats produced congestion in sinusoids, hemorrhages and vacuolation in adrenal glands. By the way, ROT was reported to have glucocorticoid-like effect as it increases the serum corticosterone in rats (Youssef *et al.,* 2003). Whether this effect mediates adrenal glands changes in ROT-treated rats, ROT effect on the adrenal gland needs further investigation. In the testes, ROT damaged seminiferous tubular epithelium, and led to loss of the sperms and cellular aggregations in some seminiferous tubules. Similarly, ROT was reported to disrupt testicular function and histology in experimental animals (Awogbindin *et al.,* 2020; Jain *et al.,* 2021). Against ROT, LiCl appeared, to some extent, alleviating ROT-induced histopathological changes in the liver, kidney, stomach, adrenal glands and testes. This effect of LiCl might be attributed to increasing the concentration of GSH and decreasing MDA formation, a lipid peroxidation marker.

Taken all together, we conclude that ROT adversely affects non-neuronal organs in rats, most notably liver, kidney, stomach, adrenal gland and testis. Against ROT, LiCl has the potential to provide observable protection against ROT-induced damage in these organs.

REFERENCES

Aebi, H. (1984): Catalase in vitro. *Methods in Enzymology, 105,* 121-126.

Ahmadi, F.A.; Linseman, D.A.; Grammatopoulos, T.N.; Jones, S.M.; Bouchard, R.J.; Freed, C.R.; Heidenreich, K.A. and Zawada, W.M. (2003): The pesticide rotenone induces caspase-3-mediated

apoptosis in ventral mesencephalic dopaminergic neurons. *Journal of Neurochemistry, 87,* 914-921.

Akinmoladun, A.C.; Oladejo, C.O.; Josiah, S.S.; Famusiwa, C.D.; Ojo, O.B. and Catechin, M.T.O. (2018): Quercetin and taxifolin improve redox and biochemical imbalances in rotenone-induced hepatocellular dysfunction: Relevance for therapy in pesticide-induced liver toxicity? *Pathophysiology, 25,* 365-371.

Ala, M.; Mohammad, Jafari, R.; Nematian, H.; Ganjedanesh, M.R.; Naderi, A.; Akbariani, M. and Dehpour, A.R. (2021): Neuroprotective Effect of Intravitreal Single-Dose Lithium Chloride after Optic Nerve Injury in Rats. *Current Eye Research, 46,* 558-567.

Alabi, A.O.; Ajayi, A.M.; Ben-Azu, B.; Bakre, A.G. and Umukoro, S. (2019): Methyl jasmonate abrogates rotenone-induced parkinsonian-like symptoms through inhibition of oxidative stress, release of pro-inflammatory cytokines and down-regulation of immnopositive cells of NF-κB and α-synuclein expressions in mice. *Neurotoxicology, 74,* 172-183.

Allen, A.L.; Luo, C.; Montgomery, D.L.; Rajput, A.H.; Robinson, C.A. and Rajput, A. (2009): Vascular pathology in male Lewis rats following short-term, low-dose rotenone administration. *Veterinary Pathology 46,* 776-782.

Awogbindin, E.O.; Adedara, I.A.; Adeniyi, P.A.; Agedah, A.E.; Oyetunde, B.F.; Olorun, P.D.; Ogbuewu, E.; Akindoyeni, I.A.; Yusuf, E.M.; Oluwatoyin, G.E.0. and Ebenezer, O.F. (2020): Nigral and ventral tegmental area lesioning induces testicular and sperm morphological abnormalities in a rotenone model of Parkinson's disease. *Environmental Toxicology and Pharmacology, 78,* 103412.

Bancroft, J.D. and Stevans, A. (1990): Theory and Practice of Histological Techniques. Churchill Livingstone, Edinburgh, 3rd ed. pp. 113-305.

Betarbet, R.; Sherer, T.B.; MacKenzie, G.; Garcia-Osuna, M.; Panov, A.V. and Greenamyre, J.T. (2000): Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature Neuroscience, 3,* 1301-1306.

Chen, G.; Bower, K.A.; Ma, C.; Fang, S.; Thiele, C.J. and Luo, J. (2004): Glycogen synthase kinase 3β (GSK3β) mediates 6-hydroxydopamine-induced neuronal death. *FASEB Journal, 18,* 1162−1164.

Degli Esposti, M. (1998): Inhibitors of NADH-ubiquinone reductase: an overview. *Biochimica Biophysica Acta, 1364,* 222–235.

Dhillon, A.S.; Tarbutton, G.L.; Levin, L.J.; Plotkin, G.M.; Lowry, L.K.; Nalbone, J.T. and Shepherd, S. (2008): Pesticide/environmental exposures and Parkinson' s disease in East Texas. *Journal of Agromedicine, 13,* 37-48.

Drolet, R.E.; Cannon, J.R.; Montero, L. and Greenamyre, J.T. (2009): Chronic rotenone exposure reproduces Parkinson's disease gastrointestinal neuropathology. *Neurobiology of Disease, 36,* 96-102.

Ellman, G.L. (1959): Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics, 82,* 70-77.

Fan, M.; Song, C.; Wang, T.; Li, L.; Dong, Y.; Jin, W. and Lu, P. (2015): Protective effects of lithium chloride treatment on repeated cerebral ischemia-reperfusion injury in mice. *Neurological Sciences, 36,* 315-321.

Gibson-Corley, K.N.; Olivier, A.K. and Meyerholz, D.K. (2013): Principles for valid histopathologic scoring in research. *Veterinary Pathology, 50,* 1-22.

Greene, J.G.; Noorian, A.R. and Srinivasan, S. (2009): Delayed gastric emptying and enteric nervous system dysfunction in the rotenone model of Parkinson's disease. Experimental Neurology, 218, 154–161.

Hassan, H.M.; Salem, D.; Badawy, A.M.; Shaaban, A.A. and ElHessy, H.M. (2023): Mitochondrial Complex I Inhibitor Class A Rotenone Induced Toxicity In Rats. Adrenal Gland And The Ameliorative Effect of Ferulic Acid Via Regulation Of Apoptosis Signaling. Journal of Pharmaceutical Negative Results, 14, 718-727.

Islam, M.B. (2006): Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, 51, 45-66.

Jain, J.; Hasan, W.; Biswas, P.; Yadav, R.S. and Jat, D. (2021): Protective Role of quercetin against rotenone-induced hepato and nephrotoxicity in Swiss albino mice. Toxicology International, 2, 57-65.

Jiang, X-W.; Qiao, L.; Feng, X-X.; Liu, L.; Wei, Q-W.; Wang, X-W. and Yu, W-H. (2017): Rotenone induces nephrotoxicity in rats: oxidative damage and apoptosis. Toxicology Mechanisms and Methods, 27, 528-536.

Johnson, M.E.; Stringer, A. and Bobrovskaya, L. (2018): Rotenone induces gastrointestinal pathology and microbiota alterations in a rat model of Parkinson's disease. NeuroToxicology, 65, 174-185.

Kumar, S.; Kumar, S.; Arthur, R. and Kumar, P. (2023): Trans-cinnamaldehyde mitigates rotenone-induced neurotoxicity via inhibiting oxidative stress in rats. Pharmacological Research - Modern Chinese Medicine, 6, 100209.

La Forge, F.B.; Haller, H.L. and Smith, L.E. (1933): The Determination of the structure of rotenone. Chemical Review, 18, 181-213.

Lapointe, N.; St-Hilaire, M.; Martinoli, M.G.; Blanchet, J.; Gould, P.; Rouillard, C. and Cicchetti, F. (2004): Rotenone induces non-specific central nervous system and systemic toxicity. FASEB J, 18,717-719.

Li, Z.S.; Schmauss, C.; Cuenca, A.; Ratcliffe, E. and Gershon, M.D. (2006): Physiological modulation of intestinal motility by enteric dopaminergic neurons and the D2 receptor: analysis of dopamine receptor expression, location, development, and function in wild-type and knock-out mice. Journal of Neuroscience, 26, 2798–2807.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951): Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193, 265-275.

Ma, J.; Gao, S.S.; Yang, H.J.; Wang, M.; Cheng, B.F.; Feng, Z.W. and Wang, L. (2018): Neuroprotective effects of proanthocyanidins, natural flavonoids derived from plants, on rotenone-induced oxidative stress and apoptotic cell death in human neuroblastoma SH-SY5Y cells. Frontier in Neuroscience, 31, 369.

Misra, H.P. and Fridovich, I. (1972): The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. Journal of Biological Chemistry, 247, 3170-3175.

Miyazaki, I.; Isooka, N.; Imafuku, F.; Sun, J.; Kikuoka, R.; Furukawa, C. and Asanuma, M. (2020): Chronic Systemic Exposure to Low-Dose Rotenone Induced Central and Peripheral Neuropathology and Motor Deficits in Mice: Reproducible Animal Model of Parkinson's Disease. International Journal of Molecular Sciences, 21, 3254.

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95,* 351-358.

Radad, K.; Hassanein, Kh.; Moldzio, R. and Rausc, W.D. (2013): Vascular damage mediates neuronal and non-neuronal pathology following short and long-term rotenone administration in Sprague-Dawley rats. *Experimental and Toxicologic Pathology, 65,* 41-47.

Sherer, T.B.; Kim, J.H.; Betarbet, R. and Greenamyre, J.T. (2003): Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. *Experimental Neurology, 179,* 9-16.

Skold, M.; Rolstad, S.; Joas, E.; Kardell, M.; Pålsson, E.; Goodwin, G.M. and Landén, M. (2021): Regional lithium prescription rates and recurrence in bipolar disorder. *International Journal of Bipolar Disorders, 9,* 18.

Tanner, C.M.; Kamel, F.; Ross, G.W.; Hoppin, J.A.; Goldman, S.M.; Korell, M.; Marras, C.; Bhudhikanok, G.S.; Kasten, M.; Chade, A.R.; Comyns, K.; Richards, M.B.; Meng, C.; Priestley, B.; Fernandez, H.H.; Cambi, F.; Umbach, D.M.; Blair, A.; Sandler, D.P. and, J.W. (2011): Rotenone, Paraquat, and Parkinson's Disease.

Environmental Health Perspectives, 119, 866-872.

Tharwat, E.K.; Abdelaty, A.O.; Abdelrahman, A.I.; Elsaeed, H.; Elgohary, A.; El-Feky, A.S.; Ebrahim, Y.M.; Sakraan, A.; Ismail, H.A.; Khadrawy, Y.A.; Aboul Ezz, H.S.; Noor, N.A.; Fahmy, H.M.; Mohammed, H.S.; Mohammed, F.F.; Radwan, N.M. and Ahmed, N.A. (2023): Evaluation of the therapeutic potential of cerebrolysin and/or lithium in the male Wistar rat model of Parkinson's disease induced by reserpine. *Metabolic Brain Disease, 38,* 1513-1529.

Wang, H.; Huo, M.; Jin, Y.; Wang, Y.; Wang, X.; Yu, W. and Jiang, X. (2022): Rotenone induces hepatotoxicity in rats by activating the mitochondrial pathway of apoptosis. *Toxicological Mechanisms and Methods, 32,* 510-517.

Wei, H.; Qin, Z.H.; Senatorov, V.V.; Wei, W.; Wang, Y.; Qian, Y. and Chuang, D.M. (2001): Lithium suppresses excitotoxicityinduced striatal lesions in a rat model of Huntington's disease. Neuroscience, 106, 603−612.

Youssef, J.; Elbi, C.; Warren, B.; Yourtee, D.; Nagarur, R.; Molteni, A.; Cunningham, M.L.; and Badr, M. (2003): Glucocorticoid-like effects of antihepatocarcinogen Rotenone are mediated via enhanced serum corticosterone levels: Molecular Fitting and Receptor Activation. *Studies J Carcinog, 14,* 2.

الآفات غير العصبية للروتينون فى جرذان سبراج داولى:

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تم ربط الروتينون والذي يعمل كمثبط للتفاعل الأول بالميتوكوندريا بمرض باركنسون منذ أن قامت الباحثة بيتاربيت وزملاؤها باستحداث معظم سمات المرض في الجرذان المعالجة بالروتينون في عام ٢٠٠٠ . والبرغم من استخدام الروتينون على نطاق واسع لنمذجة مرض باركنسون منذ ذلك الحين الا انه لا توجد تقارير مفصلة عن آثاره الضارة على الأنسجة غير العصبية. ووفقًا لذلك حاولت دراستنا الحالية القاء الضوء علي التأثير المرضي للروتينون علي الأنسجة الغير عصبية في الجرذان. وتم أيضا استكشاف التأثير الوقائي المحتمل لكلوريد الليثيوم ضد حدوث الآفات الناجمة عن الروتينون. تم توزيع أربعين جرذا ذكرًا من سلالة سبراج داولي على ٤ مجموعات كالأتي : المجموعة الضابطة والمجموعة المعالجة بالروتينون والمجموعة المعالجة بالروتينون وكلوريد الليثيوم والمجموعة المعالجة بكلوريد الليثيوم فقط. تم إعطاء الروتينون (٢ مجم/كجم من وزن الجسم) والليثيوم (٦٠ مجم/كجم من وزن الجسم) تحت الجلد وعن طريق الفم علي التوالي خمس مرات في الأسبوع لمدة ٥ أسابيع. تم تقدير أوزان الجرذان في بداية ونهاية التجربة وتقييم وظائف الجهاز الهضمي. تم ايضا الحصول على عينات الدم وعينات الأنسجة من الكبد والكلى والمعدة والغدة الكظرية والخصية. استخدمت عينات الدم في تقدير نشاط بعض الانزيمات المضادة للاكسدة. وتم تجهيز عينات الانسجة المختلفة للفحص الهستوباثولوجي. أحدث الروتينون خللا ملحوظا في وظائف الجهاز الهضمي وانخفاض نشاط الانزيمات المضادة للأكسدة وزيادة مستويات المالون داي الدهيد. تسبب الروتينون أيضا بشكل مميز في إصابة الأوعية الدموية في أعضاء الجسم المختلفة وبالتالي إتلاف أنسجة هذه الأعضاء. وفي المقابل خفف العلاج المشترك للجرذان بكلوريد الليثيوم والروتينون من معظم الآثار الضارة التي أنتجها الروتينون. خلصت الدراسة الي وجود تأثير مرضي للروتينون على الأعضاء غير العصبية في الجرذان وأبرزها الكبد والكلى والمعدة والغدة الكظرية والخصية واظهر الليثيوم كلورايد تأثيرا وقائيا ضد الضرر الناجم عن الروتينون في تلك الأعضاء .