Research Paper: Effects of Long-term Administration of *Rosemarinus Officinalis* Essential Oil on Blood Factors and Liver and Kidney Cells in Adult Rats

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ABSTRACT

Introduction: *Rosmarinus officinalis* is a useful medicinal plant with numerous ethnopharmacological applications and widespread use in the industry. The main constituent of this plant is essential oil. There is a misconception that there is no restriction to using medicinal plants. The present study examined the effects of the chronic administration of *Rosemarinus officinalis* essential oil on the liver and kidney.

Methods: Twenty-Eight Wistar rats were divided into 4 groups. Moreover, the study animals were Intraperitoneally (IP) injected for 21 days with 0.2%, 1%, and 2% *Rosemarinus officinalis* essential oil and the solvent, respectively. After this period, the study animals' blood samples were collected for renal and hepatical biochemical evaluations. Tissue samples from these organs were obtained for histomorphometric and histopathological studies.

Results: The chronic treatment of animals with *Rosemarinus officinalis* essential oil increased blood urea nitrogen and aspartate aminotransferase in the study samples' blood ($P<0.05$). The diameter of the hepatocytes distal to the central vein significantly increased, compared to the proximal ones ($P<0.05$). Atrophic and degenerative changes in the liver and kidney tissues were observed by 1% and 2% administration of *Rosemarinus officinalis* essential oil.

Conclusion: Our findings indicated that chronic and high administration of *Rosemarinus officinalis* may harm the liver and kidney.

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1. Introduction

In the last decades, the administration of herbal medicines is growing fast in numerous countries. Besides, to be abundant and affordable, they are effective in curing many diseases [1]. Not only the medicinal plants but also their bioactive constituents are used as a model for drug synthesis [2]. One of these natural components is essential oils; they are eco-friendly with various therapeutic characteristics, including anti-inflammatory, antioxidant, and anticancer activities [3, 4].

Rosemarinus officinalis, an edible evergreen shrub, from the Lamiaceae family, has long been used as a medicinal plant, for its beneficial effects [2]; most of which are proved scientifically [5]. In folk medicine, *Rosemarinus officinalis* is applied for treating the liver, kidney, intestine, and respiratory system conditions. It can also stimulate hair follicle growth and wound healing [6]. It has also been used in food industries as a spice as well as a food preservative to prevent food poisoning [7]. The most abundant component of the leaves of this plant is essential oil. Such components mainly consist of 1.8-cineole, alpha-pinene, and camphor [6, 8, 9]. A misconception indicates that medicinal plants are quite safe and without restriction in usage. Accordingly, this plant was suggested to decrease the number of primary and secondary spermatocytes and spermatids, and reduce sperm motility, and density in male rats [10].

In females, a high incidence of anomalous rat embryos, reduced the number of uterine blastocysts [11]. Furthermore, the relevant embryotoxic effects were reported in rats [12]. Additionally, this plant can inhibit the uterotropic action of estradiol and estrone [13]. The liver plays a central role in the metabolism, transport, and clearance of xenobiotics; therefore, it is highly susceptible to chemical-induced toxicity. In the West, drug-induced liver injury is a major healthcare problem and accounts for the majority of acute liver failure cases [14].

The kidney is also a common target for toxic xenobiotics. This is due to its capacity to extract and concentrate toxic substances by highly specialized cells. It could also be attributed to its large blood flow (about 21% of cardiac output) [15]. Considering the widespread use of *Rosemarinus officinalis* in the food industry and medicine, more detailed data on the toxicity of this plant seems essential. Thus, this study aimed to survey the effects of the long-term administration of *Rosemarinus officinalis* essential oil on the liver and kidney blood factors and cells in adult rats.

2. Materials and Methods

Adult *Wistar* rats weighing 180-220 gr were examined in this study. The study animals were maintained in the animal house of the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman City, Iran. The research animals were kept in plastic cages covered with wood chips. They had free access to food and water under a standard light/dark cycle. The procedure of this experiment was based on the Ethical Principles of the International Committees to protect laboratory animals (approval No. 3358, 20/07/95).

The pure leaf essential oil of *Rosemarinus officinalis* was obtained from Zardband Pharmaceuticals, Tehran City, Iran. According to the pharmaceuticals datasheet, the assay test (1,8 cineole) by BP, 2015 (GC) equaled 48.7. The study animals were randomly divided into 4 groups of 7. The study rats were Intraperitoneally (IP) injected with *Rosemarinus officinalis* essential oil for 21 days, between 11:00 AM to 3:00 PM. The concentrations of the essential oil used in this experiment for groups 1, 2, and, 3 were 0.2%, 1%, and, 2%, respectively. The LD50 value for this essential oil was equal to ≥4%. The fourth group was determined as the control and only received a solvent, containing 10% alcohol and water. During this period, all explored animals were observed daily for mortality.

On the day 21, the study rats were anesthetized and the blood samples were obtained from their hearts. The required blood samples were collected in sterile test tubes and allowed to clot for 30 min. The sera were separated by centrifuging at 4000 rpm/min for 10 min. The collected samples were stored at -20°C until assay. The biochemical indices, such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), total protein, Blood Urea Nitrogen (BUN), Creatinine Phosphokinase (CPK), and Creatinine (Cr) were analyzed using a commercial kit (Pars Azmoon Co., Tehran, Iran).

After tail collecting blood samples on the 21st day, the examined rats were sacrificed by an overdose injection of xylazine; subsequently, the samples from the liver (right lateral lobe) and the kidney (left kidney) of the study rats were obtained.

The collected samples were washed with Phosphate Buffer Saline (PBS) (pH 7) and placed in a 10% buffered

neutral formaldehyde solution (pH 7.2). After 10 days, the study samples were embedded in paraffin and fixed in 10% neutral buffered formalin solution; consequently, they were dehydrated in graded ethanol and xylol alcohols. Five-Micrometer sections (Slee® microtome; Germany) were stained with hematoxylin-eosin and assessed using a light microscope (Olympus, Japan).

For liver histomorphometry, 20 hepatical lobules were randomly selected per sample. Moreover, in each lobule, the diameter of 20 proximal and 20 distal hepatocytes located to the central vein were measured with an Olympus light microscope using a digital lens (Dino-eye, AM-7023, 5Mp, Taiwan, manufacturer part no. AM7023). Eventually, the mean value of hepatocyte diameters per sample was calculated.

For the kidney, the diameter of the bowman's capsules was subtracted from the glomerular capillary; thus, the diameter of the urinary space was obtained. In each study animal, this procedure was conducted for 20 renal corpuscles.

The obtained biochemical and histomorphometry data were analyzed by SPSS. The collected results were expressed as Mean±SEM. The differences between groups were determined by one-way Analysis of Variance (ANOVA) followed by Tukey's Post Hoc test. $P<0.05$ was considered statistically significant.

3. Results

Biochemical analysis: Our results indicated that treating animals with 0.2% *Rosemarinus officinalis* essential oil increased BUN after 21 days ($P<0.05$). Cr presented no significant effect. Among the examined liver parameters, AST demonstrated increment in treated animals with 0.2% and 2 % of *Rosemarinus officinalis* essential oil ($P<0.05$). Other factors did not significantly change (Table 1).

Histomorphometric and histopathological analysis: The obtained data suggested that in animals treated with 1% and 2% *Rosemarinus officinalis* essential oil, the diameters of hepatocytes, distal, and proximal to the central vein increased, compared to the controls ($P<0.05$) (Table 2). We detected no significant effect in the diameter of urinary space between different groups treated with *Rosemarinus officinalis* essential oil (Table 2). The liver pathological findings signified no observable change in the liver tissue among the control group and 0.2% treated animals. The normal structure of hepatocytes, such as the obvious nuclei and cytoplasms with the radial arrangments around the central vein and normal sinusoidal space were observed (Figure 1A & B). However, in 1% and 2% treated groups, congestion in the liver tissue, vacuolation, and the degenerative alteration of hepatocytes were observed (Figure 1C & D). In the kidneys of the control and 0.2% treated animals, the kidney tissue was healthy (Figure 2A & B). In the groups treated with 1% and 2%, glomerular atrophy, degeneration, and vacuolation of tubule cells, congestion around tubules, presence of inflammatory cells (neutrophils) around the vessels, and increment of urinary space were detected.

4. Discussion

According to our pilot study, the LD50 value for *Rosemarinus officinalis* essential oil was equal to 4%. Among renal characteristics, we measured BUN and creatinine. Urea nitrogen and creatinine are the byproducts of nitrogen metabolism. These are appropriate indicators of renal function. Furthermore, they are used for evaluating the functional capacity of the nephrons [16, 17]. A major function of the urinary system is to excrete BUN and creatinine. When this function is lost, plasma concentrations of Urea nitrogen and creatinine increase—a condition termed azotemia. Azotemia is the single best laboratory abnormality that indicates problems in the urinary system [18]. Our results indicated that treating animals with 0.2% *Rosemarinus officinalis* essential oil increased BUN after 21 days. In rodents, the increases in plasma urea nitrogen and creatinine occur when >75% of the renal function is lost. Other characteristics that cause increased urea nitrogen are a high protein diet and senility [18]. As in this study, the explored rats were fed with a normal standard diet, including 21% crude protein (Janveheh Khorasan, Mashhad, Iran,). Moreover, we used young but not old rats; thus, it seems that the increased urea nitrogen may be due to an abnormality in renal function by the essential oil.

Serum or plasma enzymes commonly used to detect liver disease in rodents include AP, GGT, AST, ALT, LD, and sorbitol dehydrogenase. The serum or plasma concentrations of these enzymes rise with increased production, enhanced release, or decreased clearance [18].

Aspartate aminotransferase (AST) is a mitochondrial and cytosolic enzyme with high activity in the liver, heart, skeletal muscle, and kidney as well as low activity in the intestines, brain, lung, and testes. Increases in plasma or serum AST activity are usually associated with hepatic, cardiac muscle, or skeletal muscle injuries [18]. The present study findings revealed that long-term treatment of animals with *Rosemarinus officinalis* es-

Table 1. Effect of *Rosmarinus Officinalis* essential oil on blood renal and liver biochemical parameters after 21 days

Parameters	Mean±SEM			
	Control	(Animal Groups) 0.2%	1%	2%
BUN (mg/dL)	22±1.0	28±1.0*	19±0.0	21±0.0
Cr (mg/dl)	0.42±0.01	0.48±0.01	0.32±0.0	0.45±0.05
AST (IU/L)	192±60	352±57*	93±4	443±33*
ALT (IU/L)	129±38	100±16	73±15	230±56
ALP (IU/L)	317±13	304±58	241±13	285±34

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BUN: Blood Urea Nitrogen; Cr: Creatinine; AST: Aspartate Transaminase; ALT: Alanine Transaminase; ALP: Alkaline Phosphatase. * Significant: when compared with the control group (P<0.05).

sential oil (0.2% & 2%) elevated AST. Our pathological findings supported the irritant effect of this plant on the liver and kidney. The component that makes this plant toxic remains undiscovered. Naturally, we seek our answer in the constituents of this plant. The most abundant substance in this essential oil is 1,8-cineole (48.7%). The 1,8-cineole, also named cineole or eucalyptol, is a monoterpene oxide, found in numerous plant essential oils. Conventionally, this plant is used as a food flavoring agent. It also posses antimicrobial, anti-inflammatory, anticancer, and antioxidant activity; thus, it is widely implemented for its pharmacological characteristics [19, 20]. Xu et al. explored the toxicity of 1,8-cineole [21]. They also found the adverse effects of long-term adminis-

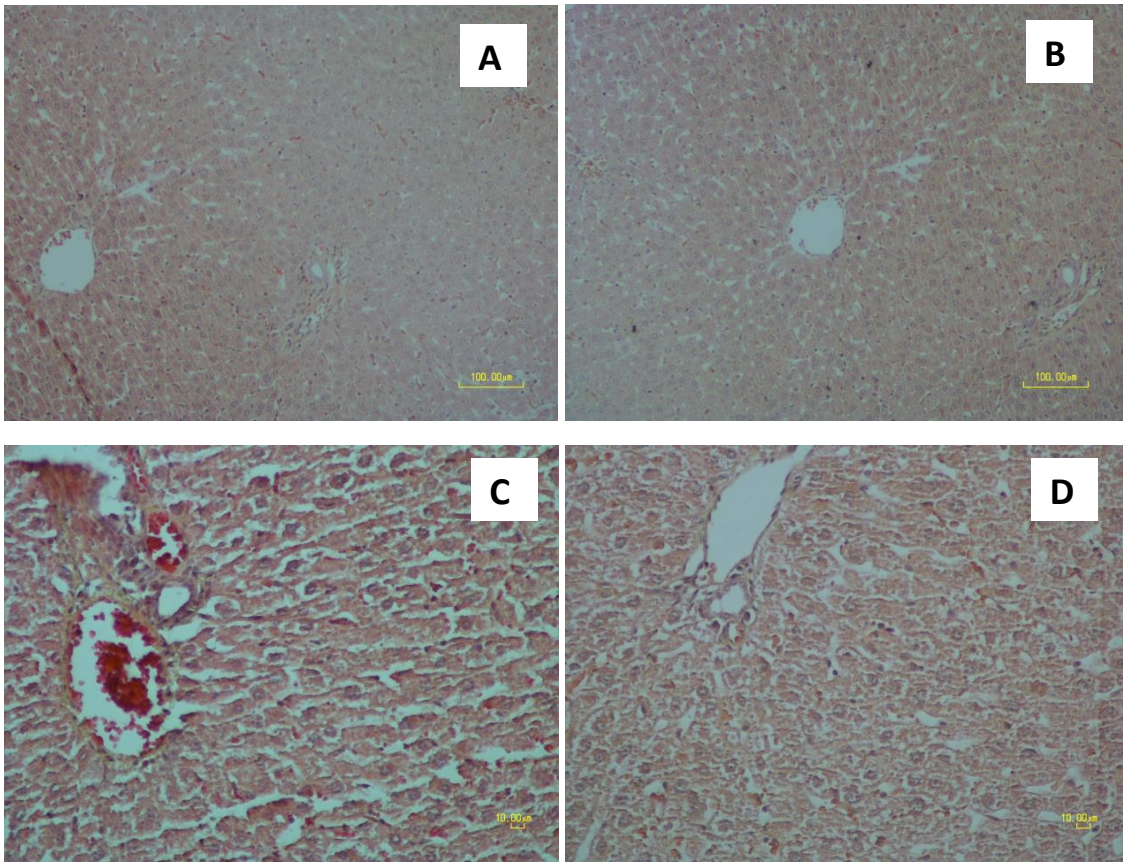

Figure 1. The histopathological evaluation of the liver

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A, B, C, and D: Show control, 0.2%, 1%, and 2% *Rosmarinus officinalis* essential oil-treated samples (H & E, A & B ×10, C& D×40).

Table 2. Effect of *Rosmarinus Officinalis* essential oil on the diameter of hepatocytes distal and proximal to the central vein, and urinary space after 21 days

Diameter (µm)	Mean±SEM			
	Control	(Animal Groups) 0.2%	1%	2%
Distal hepatocytes	8.38±0.07	8.5±0.11	9.44±0.09	9.92±0.15
Proximal hepatocytes	7.25±0.08	7.31±0.08	7.66±0.07	7.59±0.1
Urinary space	45.95±0.80	45.98±0.54	44.93±0.55	44.38±0.54

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P<0.05 is considered significant; Different letter superscripts in each row indicate the significant effect.

tration of cineol on the liver and kidney at the subcellular level. They concluded that the granular and vacuolar degenerations of these organs are related to their effect on mitochondria, endoplasmic reticulum, and other membrane-type organelles of the liver and kidney. Accordingly, maybe the effect detected in this study pertains to 1,8-cineole. Another similar study examined the chronic administration of (30 days) cineole-induced histological alterations in the lungs, liver, kidneys, and uterus; they caused maternal and fetal toxicity in pre-implantation or organogenesis. In the mentioned study, consistent with our study, the urea level increased. It highlights that this compound can also affect other organs [22].

5. Conclusion

The current research data indicated that the long-term administration of *Rosmarinus officinalis* may presnt some adverse effects on the liver and kidney. The leaves of this plant are commonly used as a spice or flavoring

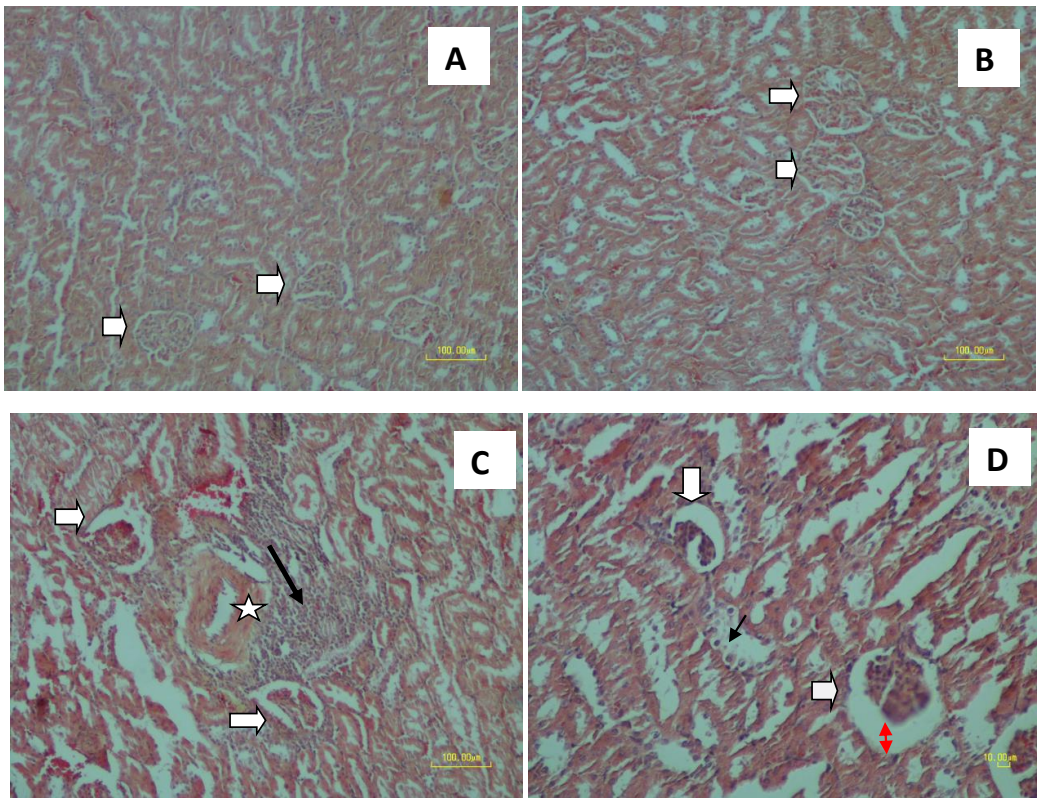

Figure 2. The effects of *Rosmarinus officinalis* essential oil on the kidney tissue

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The white arrows per figure illustrate the glomerular corpuscle. A: Related to the control group; B: Shows the tissue in a 0.2% percent treated animal; C: The star signifies a vessel surrounded by the neutrophils (the black arrow) in a 1% essential oil-treated animal; D: The black arrow reflects the vacuolation and degeneration of tubule cells. The red arrow indicates the urinary space increment (H & E, A, B & C ×10, D ×40).

agent. Moreover, as an evergreen plant, it may be used by domestic animals. Thus, its expenditures should be more cautiously considered.

Ethical Considerations

Compliance with ethical guidelines

The procedure of this experiment was based on the Ethical Principles of the International Committees to protect laboratory animals (Approval No. 3358, 20/07/95).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflicts of interest.

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References

[1] Bone K, Mills S. Principles and practice of phytotherapy: Modern herbal medicine. London: Churchill Livingstone; 2000. https://books.google.com/books?id=-mIuywEACAAJ&dq

[2] Andrade JM, Faustino C, Garcia C, Ladeiras D, Reis CP, Rijo P. *Rosmarinus officinalis* L.: An update review of its phytochemistry and biological activity. Future Science OA. 2018; 4(4):FSO283. [DOI:10.4155/fsoa-2017-0124] [PMID] [PMCID]

[3] Borah A, Paw M, Gogoi R, Loying R, Sarma N, Munda S, et al. Chemical composition, antioxidant, anti-inflammatory, anti-microbial and *in-vitro* cytotoxic efficacy of essential oil of *Curcuma caesia Roxb.* leaves: An endangered medicinal plant of North East India. Industrial Crops and Products. 2019; 129:448-54. [DOI:10.1016/j.indcrop.2018.12.035]

[4] Miguel MG. Antioxidant and anti-inflammatory activities of essential oils: A short review. Molecules. 2010; 15(12):9252-87. [DOI:10.3390/molecules15129252] [PMID] [PMCID]

[5] Ribeiro-Santos R, Carvalho-Costa D, Cavaleiro C, Costa HS, Albuquerque TG, Castilho MC, et al. A novel insight on an ancient aromatic plant: The rosemary (*Rosmarinus officinalis* L.). Trends in Food Science & Technology. 2015; 45(2):355-68. [DOI:10.1016/j.tifs.2015.07.015]

[6] Zargari A. Medicinal Plants. Vol. 3. Tehran: University of Tehran Press; 1997. https://www.google.com/books/edition/Medicinal_plants/SPdJnQEACAAJ?hl=en

[7] Joshi SC, Sharma A, Chaturvedi M. Antifertility potential of some medicinal plants in males: An overview. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(5):204-17. https://innovareacademics.in/journal/ijpps/Vol3Suppl5/2761.pdf

[8] Tak JH, Jovel E, Isman MB. Comparative and synergistic activity of *Rosmarinus officinalis* L. essential oil constituents against the larvae and an ovarian cell line of the cabbage looper, Trichoplusia ni (Lepidoptera: Noctuidae). Pest Management Science. 2016; 72(3):474-80. [DOI:10.1002/ps.4010] [PMID]

[9] Kladniew BR, Polo M, Villegas SM, Galle M, Crespo R, de Bravo MG. Synergistic antiproliferative and anticholesterogenic effects of linalool, 1, 8-cineole, and simvastatin on human cell lines. Chemico-Biological Interactions. 2014; 214:57-68. [DOI:10.1016/j.cbi.2014.02.013] [PMID]

[10] Heidari Vala H, Ebrahimi Hariry R, Sadeghi MR, Akhondi MM, Ghaffari Novin M, Heidari M. Evaluation of an aqueous-ethanolic extract from Rosmarinus officinalis (Rosemary) for its activity on the hormonal and cellular function of testes in adult male rat. Iranian Journal of Pharmaceutical Research. 2013; 12(2):445-51. [DOI:10.22037/IJPR.2013.1307] [PMID] [PMCID]

[11] Damasco DC, Lemonica IP. Embryotoxicity and anti-implantation effects of Rosemary (Rosmarinus officinalis L.) extract in pregnant rats within preimplantation period. Revista Brasileira de Toxicologia. 1999; 12(2):47-54. https://pesquisa.bvsalud.org/portal/resource/pt/lil-282947

[12] Lemonica IP, Damasceno DC, di-Stasi LC. Study of the embryotoxic effects of an extract of rosemary (*Rosmarinus officinalis* L.). Brazilian Journal of Medical and Biological Research. 1996; 29(2):223-7. [PMID]

[13] Zhu BT, Loder DP, Cai MX, Ho CT, Huang MT, Conney AH. Dietary administration of an extract from rosemary leaves enhances the liver microsomal metabolism of endogenous estrogens and decreases their uterotropic action in CD-1 mice. Carcinogenesis. 1998; 19(10):1821-7. [DOI:10.1093/carcin/19.10.1821] [PMID]

[14] Corsini A, Bortolini M. Drug-induced liver injury: The role of drug metabolism and transport. The Journal of Clinical Pharmacology. 2013; 53(5):463-74. [DOI:10.1002/jcph.23] [PMID]

[15] Martinez-Salgado C, López-Hernández FJ, López-Novoa JM. Glomerular nephrotoxicity of aminoglycosides. Toxicology and Applied Pharmacology. 2007; 223(1):86-98. [DOI:10.1016/j.taap.2007.05.004] [PMID]

[16] Begum A, Sandhya S, Ali SS, Vinod KR, Reddy S, Banji D. An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). Acta Scientiarum Polonorum Technologia Alimentaria. 2013; 12(1):61-73. [PMID]

[17] Tabarraei H, Hassan J, Parvizi MR, Golshahi H, Keshavarz-Tarikhi H. Evaluation of the acute and sub-acute toxicity of the black caraway seed essential oil in Wistar rats. Toxicology

Reports. 2019; 6:869-74. [DOI:10.1016/j.toxrep.2019.08.010] [PMID] [PMCID]

[18] Thrall MA, Weiser G, Allison RW, Campbell TW. Veterinary hematology and clinical chemistry. 2nd edition. Hoboken: John Wiley & Sons; 2012. https://books.google.com/books?id=PjCanfyADvIC&printsec=frontcover&dq

[19] Juergens LJ, Worth H, Juergens UR. New perspectives for mucolytic, anti-inflammatory and adjunctive therapy with 1, 8-cineole in COPD and asthma: Review on the new therapeutic approach. Advances in Therapy. 2020; 37(5):1737-53. [DOI:10.1007/s12325-020-01279-0] [PMID] [PMCID]

[20] Dhakad AK, Pandey VV, Beg S, Rawat JM, Singh A. Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: A review. Journal of the Science of Food and Agriculture. 2018; 98(3):833-48. [DOI:10.1002/jsfa.8600] [PMID]

[21] Xu J, Hu ZQ, Wang C, Yin ZQ, Wei Q, Zhou LJ, et al. Acute and subacute toxicity study of 1, 8-cineole in mice. International Journal of Clinical and Experimental Pathology. 2014; 7(4):1495-501. [PMID] [PMCID]

[22] Caldas GFR, Limeira MMF, Araújo AV, Albuquerque GS, da Costa Silva-Neto J, da Silva TG, et al. Repeated-doses and reproductive toxicity studies of the monoterpene 1, 8-cineole (eucalyptol) in Wistar rats. Food and Chemical Toxicology. 2016; 97:297-306. [DOI:10.1016/j.fct.2016.09.020] [PMID]

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