Improvement of Renal Proximal Tubules after Black Cumin (Nigella Sativa) Extract Administration in Rat with CCl4-induced Chronic Renal Damage

Fathiyah Safithri[1,*], Desy Andari[2] and Fifa Yuniarmi
[1]*Department of Pharmacology, Faculty of Medicine, University of Muhammadiyah Malang Jalan Bendungan Sutami No. 188A, Malang, East Java, Indonesia*
[2]*Histology Department Faculty of Medicine, University of Muhammadiyah Malang, Jalan Bendungan Sutami No. 188A, Malang, East Java, Indonesia*

Keywords: Black cumin extract, hyaline cast tubular, chronic renal damage, CCl_4.

Abstract: Chronic renal disease is a chronic pathological process in renal that accompanied by a progressive decline in renal function and generally ends up as renal failure. Chronic inflammation and oxidative stress are key factors in progression. Black cumin extract has antioxidant and anti-inflammatory effects that is thought to repair renal tubular and inhibit progression of the damage. Objectives: to evaluate the improvement of renal proximal tubules in rat with CCl4-induced chronic renal damage after black cumin extract administration. Results: The result showed that the amount of hyaline cast in the proximal tubule significantly decreased after black cumin extract adminstration. Conclusion: Black cumin extract decreases the amount of hyaline cast in the proximal tubule. The antioxidant and anti-inflammatory effects of Black cumin extract may modulate the improvement renal tubular.

SCITEPRESS SCIENCE AND TECHNOLOGY PUBLICATIONS

1 INTRODUCTION

The improvement of renal function occurs in patients with chronic renal disease who consume black cumin extract (Nigella sativa) (Ansari, Nasiruddin, Khan, & Haque, 2016). Chronic renal disease is defined as abnormalities in the structure or function of the renal that last more than three months (Webster, Nagler, Morton, & Masson, 2017). The prevalence of chronic kidney disease worldwide is around 11-13% (Hill et al., 2016). Annually about 1.5% of patients with stage 3 and 4 chronic renal disease will progress to grade 5 or end stage chronic renal disease (renal failure) (Webster et al., 2017).

Chronic kidney disease is a multifactorial disease. In developed countries, the most common cause is diabetic nephropathy, while in developing countries the most common cause is chronic glomerulonephritis and intestinal nephritis. Some risk factors for chronic kidney disease are hypertension, diabetes mellitus, urinary tract infections, urinary tract stones, age-related congenital kidney disease, family history of chronic kidney disease, obesity, cardiovascular disease, low birth weight, autoimmune diseases such as systemic lupus erythematosus (SLE)) and drug poisoning or other toxicity (Gunatilake, Seneff, & Orlando, 2019).

Injury mechanism due to toxicity to the kidneys varies and all renal structures will be affected. The tubulointerstitial compartment is the compartment most commonly involved (Shirali & Perazella, 2014). Tubular epithelial cells are very sensitive to anoxia and susceptible to toxins (Berger & Moeller, 2014). Injury due to toxic substances in renal will activate macrophages to stimulate the release of cytokines such as IL-1β, IFN-γ and TNF-α (Akchurin & Kaskel, 2015). Continouse inflammation of the renal progressively causes severe kidney damage and eventually end up as chronic renal failure. The proximal tubules damage is characterized by narrowing of the tubules, epithelial necrosis and the presence of hyaline cast. Hyaline cast is a glycoprotein matrix derived from renal tubular epithelial cells that shows an abnormal condition in the renal tubules (Caleffi & Lippi, 2015).

Management of chronic renal failure includes conservative therapy to prevent progressive

Safithri, F., Andari, D. and Yuniarmi, F.
Improvement of Renal Proximal Tubules after Black Cumin (Nigella Sativa) Extract Administration in Rat with CCl4-induced Chronic Renal Damage.
DOI: 10.5220/0009120000210026
In *Proceedings of the 2nd Health Science International Conference (HSIC 2019)*, pages 21-26
ISBN: 978-989-758-462-6
Copyright © 2020 by SCITEPRESS – Science and Technology Publications, Lda. All rights reserved

deterioration in renal function, symptomatic therapy and renal replacement therapy. Pharmacological therapy is also provided to control chronic inflammation as a key factor in the progression of chronic renal damage. Another approach for repairing the tubular renal damage is reducing proinflammatory mediators with natural materials, such as black cumin seed (Yimer, Tuem, Karim, Ur-Rehman, & Anwar, 2019).

Black cumin (Nigella sativa) is one of the medicinal plants included in the Ranunculaceae family, has long been known and used as a medicinal plant. Black cumin contains more than 100 bioactives (multiple compounds) and not all bioactive activities are known (Karaçil Ermumucu & Şanlıer, 2017). The main components of black cumin oil contained in many seeds that are thought to act as anti-inflammatory are thymoquinone, carvacrol, saponin, oleic acid and linoleic acid (Parandin, Yousofvand, & Ghorbani, 2012). Invivo research by Keyhanmanesh in 2010 showed that the administration of thymoquinone in pigs with pulmonary inflammation had an effect in the form of improving the picture of the structure of pulmonary histology through a decrease in IFN-γ (Keyhanmanesh, Boskabady, Khamneh, & Doostar, 2010). Other studies have shown that carvacrol given to mice that are inflamed on their fingers has an improved effect through decreased IL-1β production (Lima et al., 2013). Giving total saponins from ginseng in pigs that have myocardial injury is proven to reduce the IL-1β proinflammatory mediator so that there is repair in damaged areas (Aravinthan et al., 2015). Other studies also prove that the administration of black cumin which has the main content of linoleic acid and oleic acid can reduce the production of IL-1β and TNF-α in mice induced by dimethylbenzantresana so as to improve the lung cells of damaged mice (Rahayu, Achmad, & Ekowati, 2012).

Based on that phenomena, the researchers want to prove whether the extract of black cumin seeds which has anti-inflammatory and anti-oxidant properties can reduce proximal tubular damage in rats model of chronic kidney damage using CCl_4. This research was conducted to prove the curative effect of black cumin on tubular damage after CCl4 exposure. Previous research on black cumin has been more on the protective or preventive effects of black cumin.

3 RESULTS AND DISCUSSION

Figure 1 show the hyaline cast tubular each group. In A group /the normal groups (without induced CCl4 and black cumin seed extract), the proximal tubules appear normal, characterized by intact cuboidal epithelium, a small number of necrotic cells in the form of cells that have been lysis (lost) and a little hyaline cast in the middle of the lumen. In B group, the positive control group (only given CCl4 1ml / kg BW / day without giving black cumin), the epithelium of the proximal tubule cells appears irregular and intact, the necrosis cells appear more than normal cells and hyaline cast which is in the middle of the lumen more than with normal groups.

In C group (induced CCl4 + black cumin seed extract) 1.2 g/kg BW/day), visible proximal tubular cuboid epithelium began to be intact, necrotic cells in the form of cariolysis and cell lysis (lost), reduced, hyaline less cast than the positive control group. In D group (induced CCl4 + black cumin extract 2.4 g/kg BW/day), the proximal tubule cuboid epithelium began to appear intact, more normal cells than the positive control group and C group, still obtained necrosis cells in the form of cariolysis and cell lysis (lost) is reduced and hyaline cast in the middle of the lumen.

In E group (induced CCl4 + black cumin extract 4.8 g/kg BW/day), the proximal tubule cuboid epithelium began to appear intact, more normal cells than the positive control group, C and D groups, still found necrotic cells in the form of cariolysis and cell lysis (lost) is reduced and hyaline cast in the middle of the lumen is approaching a normal group.

Table 1: The mean of amount hyaline cast tubular in normal rat (A), rats induced CCl4 (B), and rat-induced CCl_4 and black cumin extract administration (C, D, E).

	The mean of amount *hyaline cast* tubular (mean±SD)
A. Normal	1.70±0.65
B. CCl_4 control 1 ml/kgBB)	6.33±1.14
C. (CCl_4 1 ml/kgBB + black cumin extract 1.2 g/kgBB)	4.97±1.10

D. (CCl_4 1 ml/kgBB + black cumin extract 2.4 g/kgBB)	3.13±1.09
E. (CCl_4 1 ml/kgBB + black cumin extract 4.8 g/kgBB)	2.10±0.87

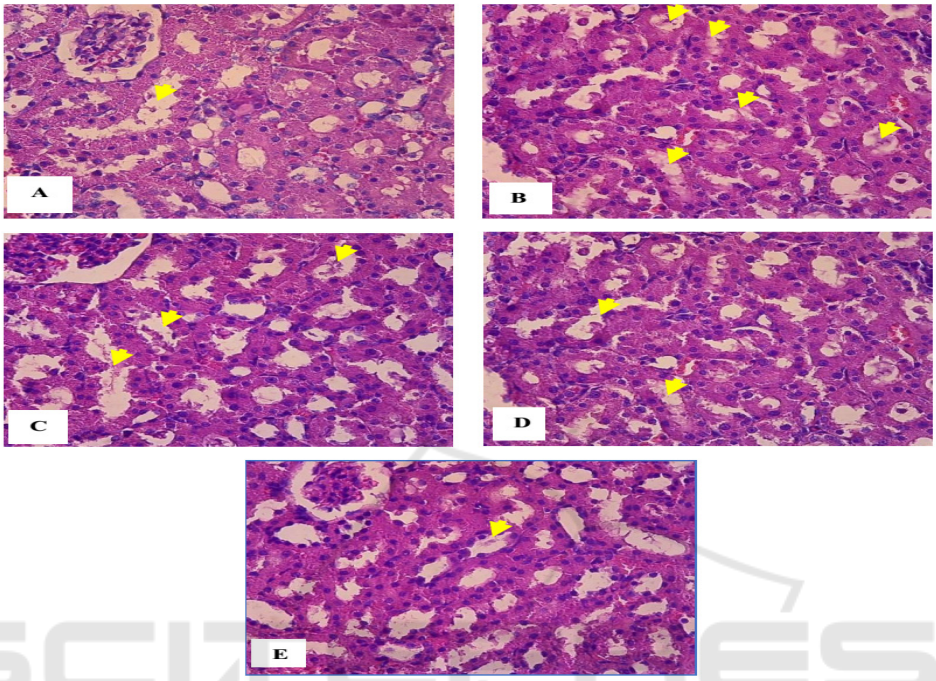

Figure 1. The hyaline cast tubular in normal rats (A), rats-induced CCl4 (B), and rats-induced CCl4 and black cumin administration in dose 1,2; 2,4; 4,8 g/kgBB (C, D, and E) at 400x magnification. The yellow arrows show positive hyaline cast tubular, marked as pink color on the tubular lumen.

The mean amount of hyaline cast tubular from all groups are explaines in Table 1. CCl4 induction significantly increases the mean amount of hyaline cast tubular ($p<0,05$). Black cumin extract administration in dose 1.2; 2.4; 4.8 g/kgBB) significantly reduces the mean amount of hyaline cast tubular ($p<0,05$).

In this study CCl_4 administration at a dose of 1 ml / kgBW 3 times a week for 8 weeks was proven to cause kidney damage which was characterized by increasing the average number of hyaline casts. Previous studies of 1 ml / kg body weight of CCl_4 administered intraperitoneally three times a week for 8 weeks were also shown to cause damage to the rat kidney, tubal dilatation, and appearance of foamy epithelial cells in the tubular area. Kidney damage is caused by toxic exposure to CCl_4 which is toxic (Venkatanarayana, Sudhakara, Sivajyothi, & Indira, 2012). CCl_4 in the body will undergo a process of biotransformation by the CYP2E1 enzyme forming free radicals namely trichormormyl radical (CCl_3). These radicals will then react with oxygen (Khan, Khan, & Sahreen, 2012).

The result of trichlormethyl radical reaction with oxygen will cause trauma, lesions and inflammation in the proximal tubules of mice so that it stimulates pro-inflammatory mediators of T lymphocytes and Natural Killer cells to produce INF-γ. IFN-γ will then activate macrophages, these macrophages will produce TNF-α and IL-1β, then an inflammatory cascade will arise. This ongoing inflammation will cause damage to the proximal tubule (El Boshy et al., 2017)

In this situation, damage to the proximal tubule will cause an increase in the permeability of the glomerular membrane, thus allowing protein (albumin) and substances dissolved in the plasma bound to the protein to easily pass through it. Increased permeability of the glomerular membrane and disruption of the function of the proximal tubule reabsorbtion cause an increase in protein in the urine

causing hypoalbumin in the body (Dickson, Wagner, Sandoval, & Molitoris, 2014).

In the histopathological picture of proximal tubular damage one of which is characterized by an increase in the average number of hyaline cast. Hyaline casts are formed from several components namely renal tubular epithelial cells, neutrophils, eosinophils, which will stick to the surface or enter the Tamm-Horsfall protein, where the Tamm-horsfall protein is a glycoprotein matrix which is sticky and only secreted by the proximal tubule. The attachment of tubular renal components to the epithelial cells, neutrophils, eosinophils on the surface of the tamm-horsfall protein will cause the formation of semi-solid lumps in the lumen and the HE staining will appear pink, where in normal circumstances no hyaline cast formation is found because the process does not occur (Enhancement, 2012).

In this study the administration of black cumin seed extract (Nigella sativa) at a dose of 1.2 g / kgBB / day, 2.4 g / kgBB / day, 4.8 g / kgBB / day can reduce the average number of hyaline cast. The reduction in the mean of amount hyaline cast tubular is thought to be caused by the active compound content of black cumin seed extract (Nigella sativa) which has anti-inflammatory and antioxidant effect.The main components in black cumin extract that have antiinflammatory effect are thymoquinone, carvacrol, linoleic acid, oleic acid and saponins which can inhibit pro-inflammatory mediators such as TNF-α, IL-1β and IFN-γ (Hadi, Kheirouri, Alizadeh, Khabbazi, & Hosseini, 2016) . Inhibition of the mediator causes the formation of an inflammatory cascade that can cause damage to the kidney tubular epithelial cells and the emergence of various macrophages that function as the main components of hyaline cast formation.

Previous studies have shown that the administration of thymoquinone to inflammatory pigs' lungs by ovalbumin induction provides an improved effect on the structure of pulmonary histology by decreasing IFN-gamma (Keyhanmanesh et al., 2010). Other studies have also shown that carvacrol given to mice that have inflammation in their fingers has an improved effect through decreased IL-1β production (Lima et al., 2013). Administration of the total saponin contained in ginseng in pigs that have myocadial injury can reduce proinflammatory mediators such as IL-1β so as to repair damaged cells (Aravinthan et al., 2015). Other studies also prove that the administration of black cumin has the main content of linoleic acid and oleic acid can reduce the production of IL-1β and TNF-α in mice induced by dimethylbenz antresana so as to improve the lung cells of damaged mice (Rahayu et al., 2012). In another study it was proven that the saponin content contained in Asian ginseng extract could increase the number of new blood vessels (angiogenesis) in the mandibular socket of mice after tooth extraction via VEGF. An increase in the number of new blood vessels can increase the process of regeneration and repair of renal tubular cells (Ahmad et al., 2013).

Black cumin or black cumin (Nigella sativa L) contains several active compounds that have antioxidant effect, the most important of which are thymoquinone (30% -48%), thymohydroquinone, dithymoquinone, p-cymene (7% -15%), carvacrol (6% -12%)), 4-terpineol (2% -7%), t-anethol (1% - 4) (Ahmad et al., 2013). Thymoquinone has a protective effect on cells against damage caused by oxidative stress (Abdelmeguid, Fakhoury, Kamal, & Al Wafai, 2010). Thymoquinone works to inhibit oxidative stress by increasing the activity of the enzyme Super Oxide Demutase (SOD), the enzyme glutathione and inhibits the lipid peroxidase reaction (Leong, Rais Mustafa, & Jaarin, 2013) (Goyal et al., 2017). Thymoquinone has been shown to significantly reduce colonic MDA levels in mice by induction of the Necrotizing enterocolitis (NEC) model (Tayman et al., 2012). Likewise in a study conducted by Fouda, 2008 proved that Thymoquinone inhibits increased MDA levels in the kidneys HgCl2-induced mice (Fouda, Daba, Dahab, & Sharaf El-Din, 2008). Thymoquinone reduced renal levels of MDA that were induced by gentamicin (Samarghandian, Azimi-Nezhad, Mehrad-Majd, & Mirhafez, 2015). In addition, black cumin also contains flavonoids and fatty acids including linoleic acid and oleic acid. Linoleic acid and oleic acid have antioxidant effects that can inhibit the lipid peroxidase reaction (El-Agbar, Naik, & Shakya, 2018). While flavonoids function as antioxidants by acting as electron donors which are targets of free radicals [(Banjarnahor & Artanti, 2014).

The dosage of black cumin seed extract (Nigella sativa.) Which gives a significant influence on the improvement of proximal tubules in the kidney of male white rats model of chronic kidney damage is a dose of 2.4 gr/kgBB/day and 4.8 gr/kgBB/day. There is a very strong and inversely related relationship between administration of black cumin extract (Nigella sativa) to the improvement of proximal tubules in the kidney of male white rats, a model of chronic kidney damage was shown by Pearson correlation test = -0.829.

4 CONCLUSIONS

Black cumin extract decreases the amount of hyaline cast in the proximal tubule. The antioxidant and anti-inflammatory effects of Black cumin extract may modulate the improvement renal tubular.

ACKNOWLEDGEMENTS

The autors thanks Miftachurrahman, Slamet, and Anto for their dedicated work in collecting data used in this article as a part of the objective of our research.

REFERENCES

Abdelmeguid, N. E., Fakhoury, R., Kamal, S. M., & Al Wafai, R. J. (2010). Effects of Nigella sativa and thymoquinone on biochemical and subcellular changes in pancreatic β-cells of streptozotocin-induced diabetic rats. *Journal of Diabetes*, *2*(4), 256–266. https://doi.org/10.1111/j.1753-0407.2010.00091.x

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A., … Anwar, F. (2013). A review on therapeutic potential of Nigella sativa: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine*, *3*(5), 337–352. https://doi.org/10.1016/S2221-1691(13)60075-1

Akchurin, O. M., & Kaskel, F. (2015). Update on inflammation in chronic kidney disease. *Blood Purification*, *39*(1–3), 84–92. https://doi.org/10.1159/000368940

Ansari, Z. M., Nasiruddin, M., Khan, R. A., & Haque, S. F. (2016). *EVALUATION OF EFFICACY AND SAFETY OF NIGELLA SATIVA OIL SUPPLEMENTATION IN PATIENTS OF CHRONIC KIDNEY DISEASE*. *9*(2).

Aravinthan, A., Kim, J. H., Antonisamy, P., Kang, C. W., Choi, J., Kim, N. S., & Kim, J. H. (2015). Ginseng total saponin attenuates myocardial injury via anti-oxidative and anti-inflammatory properties. *Journal of Ginseng Research*, *39*(3), 206–212. https://doi.org/10.1016/j.jgr.2014.12.001

Banjarnahor, S. D. S., & Artanti, N. (2014). Antioxidant properties of flavonoids. *Medical Journal of Indonesia*, *23*(4), 239–244. https://doi.org/10.13181/mji.v23i4.1015

Berger, K., & Moeller, M. J. (2014). Mechanisms of epithelial repair and regeneration after acute kidney injury. *Seminars in Nephrology*, *34*(4), 394–403. https://doi.org/10.1016/j.semnephrol.2014.06.006

Caleffi, A., & Lippi, G. (2015). Cylindruria. *Clinical Chemistry and Laboratory Medicine*, *53*(June), S1471–S1477. https://doi.org/10.1515/cclm-2015-0480

Dickson, L. E., Wagner, M. C., Sandoval, R. M., & Molitoris, B. A. (2014). The proximal tubule and albuminuria: Really! *Journal of the American Society of Nephrology*, *25*(3), 443–453. https://doi.org/10.1681/ASN.2013090950

El-Agbar, Z. A., Naik, R. R., & Shakya, A. K. (2018). Fatty acids analysis and antioxidant activity of fixed oil of quercus infectoria, grown in Jordan. *Oriental Journal of Chemistry*, *34*(3), 1368–1374. https://doi.org/10.13005/ojc/340324

El Boshy, M. E., Abdelhamidb, F., Richab, E., Ashshia, A., Gaitha, M., & Qustya, N. (2017). Attenuation of CCl4 Induced Oxidative Stress, Immunosuppressive, Hepatorenal Damage by Fucoidan in Rats. *Fermentation Technology*, *07*(03). https://doi.org/10.4172/2167-7972.1000348

Enhancement, S. E. (2012). *SEED Urinalysis*. (February).

Fouda, A. M. M., Daba, M. H. Y., Dahab, G. M., & Sharaf El-Din, O. A. (2008). Thymoquinone ameliorates renal oxidative damage and proliferative response induced by mercuric chloride in rats. *Basic and Clinical Pharmacology and Toxicology*, *103*(2), 109–118. https://doi.org/10.1111/j.1742-7843.2008.00260.x

Goyal, S. N., Prajapati, C. P., Gore, P. R., Patil, C. R., Mahajan, U. B., Sharma, C., … Ojha, S. K. (2017). Therapeutic potential and pharmaceutical development of thymoquinone: A multitargeted molecule of natural origin. *Frontiers in Pharmacology*, *8*(SEP), 1–19. https://doi.org/10.3389/fphar.2017.00656

Gunatilake, S., Seneff, S., & Orlando, L. (2019). Glyphosate's Synergistic Toxicity in Combination with Other Factors as a Cause of Chronic Kidney Disease of Unknown Origin. *International Journal of Environmental Research and Public Health*, *16*(15), 2734. https://doi.org/10.3390/ijerph16152734

Haddad, P. S., Benhaddou-Andaloussi, A., Martineau, L., Vuong, T., Meddah, B., Madiraju, P., & Settaf, A. (2011). The in vivo antidiabetic activity of Nigella sativa is mediated through activation of the AMPK pathway and increased muscle Glut4 content. *Evidence-Based Complementary and Alternative Medicine*, *2011*. https://doi.org/10.1155/2011/538671

Hadi, V., Kheirouri, S., Alizadeh, M., Khabbazi, A., & Hosseini, H. (2016). Effects of Nigella sativa oil extract on inflammatory cytokine response and oxidative stress status in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled clinical trial. *Avicenna Journal of Phytomedicine*, *6*(1), 34–43. https://doi.org/10.22038/ajp.2016.3910

Hill, N. R., Fatoba, S. T., Oke, J. L., Hirst, J. A., Callaghan, A. O., Lasserson, D. S., & Hobbs, F. D. R. (2016). Global Prevalence of Chronic Kidney Disease – A Systematic Review and Meta-Analysis. *PLoS ONES ONE*, *11*(7), 1–18. https://doi.org/10.5061/dryad.3s7rd.Funding

Karaçil Ermumucu, M. Ş., & Şanlıer, N. (2017). BLACK CUMIN (Nigella sativa) AND ITS ACTIVE COMPONENT OF THYMOQUINONE: EFFECTS

ON HEALTH. *Journal of Food and Health Science*, (January), 170–183. https://doi.org/10.3153/jfhs17020

Keyhanmanesh, R., Boskabady, M. H., Khamneh, S., & Doostar, Y. (2010). Effect of thymoquinone on the lung pathology and cytokine levels of ovalbumin-sensitized guinea pigs. *Pharmacological Reports*, *62*(5), 910–916. https://doi.org/10.1016/S1734-1140(10)70351-0

Khan, R. A., Khan, M. R., & Sahreen, S. (2012). CCl4-induced hepatotoxicity: Protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. *BMC Complementary and Alternative Medicine*, *12*, 2–7. https://doi.org/10.1186/1472-6882-12-178

Leong, X. F., Rais Mustafa, M., & Jaarin, K. (2013). Erratum: Nigella sativa and Its Protective Role in Oxidative Stress and Hypertension (Evidence-based Complementary and Alternative Medicine). *Evidence-Based Complementary and Alternative Medicine*, *2013*. https://doi.org/10.1155/2013/253479

Lima, M. D. S., Quintans-Júnior, L. J., De Santana, W. A., Martins Kaneto, C., Pereira Soares, M. B., & Villarreal, C. F. (2013). Anti-inflammatory effects of carvacrol: Evidence for a key role of interleukin-10. *European Journal of Pharmacology*, *699*(1–3), 112–117. https://doi.org/10.1016/j.ejphar.2012.11.040

Parandin, R., Yousofvand, N., & Ghorbani, R. (2012). The enhancing effects of alcoholic extract of Nigella sativa seed on fertility potential, plasma gonadotropins and testosterone in male rats. *Iranian Journal of Reproductive Medicine*, *10*(4), 355–362.

Rahayu, W. P., Achmad, A., & Ekowati, H. (2012). Aktivitas Antiproliferatif Jintan Hitam (Nigell sativa) pada Sel Paru Tikus yang Diinduksi 7,12-Dimetilbenz[a]Antrasena(DMBA). *Makara, Kesehatan*, *16*(2), 51–56.

Samarghandian, S., Azimi-Nezhad, M., Mehrad-Majd, H., & Mirhafez, S. R. (2015). Thymoquinone Ameliorates Acute Renal Failure in Gentamicin-Treated Adult Male Rats. *Pharmacology*, *96*(3–4), 112–117. https://doi.org/10.1159/000436975

Shirali, A. C., & Perazella, M. A. (2014). Tubulointerstitial Injury Associated With Chemotherapeutic Agents. *Advances in Chronic Kidney Disease*, *21*(1), 56–63. https://doi.org/10.1053/j.ackd.2013.06.010

Tayman, C., Cekmez, F., Kafa, I. M., Canpolat, F. E., Cetinkaya, M., Uysal, S., … Sarici, S. U. (2012). Beneficial effects of nigella sativa oil on intestinal damage in necrotizing enterocolitis. *Journal of Investigative Surgery*, *25*(5), 286–294. https://doi.org/10.3109/08941939.2011.639849

Venkatanarayana, G., Sudhakara, G., Sivajyothi, P., & Indira, P. (2012). Protective effects of curcumin and vitamin E on carbon tetrachloride-induced nephrotoxicity in rats. *EXCLI Journal*, *11*, 641–650. https://doi.org/10.17877/DE290R-5143

Webster, A. C., Nagler, E. V., Morton, R. L., & Masson, P. (2017). Chronic Kidney Disease. *The Lancet*, Vol. 389, pp. 1238–1252. https://doi.org/10.1016/S0140-6736(16)32064-5

Yimer, E. M., Tuem, K. B., Karim, A., Ur-Rehman, N., & Anwar, F. (2019). Nigella sativa L. (Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses. *Evidence-Based Complementary and Alternative Medicine*, *2019*. https://doi.org/10.1155/2019/1528635

SCITEPRESS SCIENCE AND TECHNOLOGY PUBLICATIONS