

Changes in Hematological Parameters of Sprague Dawley Rats with Use of Cyclosporine and Nigella Sativa

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Abstract

Objective: The objective of this study was to observe the signs of Cyclosporine toxicity and to analyze the ameliorative effects of Nigella Sativa (NS or Kalonji) on body weight and hematological parameters.

Methods: This study was conducted in the department of Anatomy, Baqai Medical University of Karachi during the month of February 2017. Thirty healthy male Sprague Dawley rats were selected from the Animal House of the university. After acclimatization, the animals were randomly divided into three Groups, with 10 rats in each Group. Group A was control group and received no intervention. Group B received treatment of oral Cyclosporine (15 mg/kg/day) in a single daily dose and Group C was given Cyclosporine (15 mg/kg/day) with Nigella Sativa seeds (450 mg/kg) as a protective agent. The body weight of rats in each Group was measured at the start of the study period. The treatment was given for the duration of 21 days, after which the body weight was measured again. The rats were anesthetized and blood was collected through cardiac puncture. Blood analysis for hemoglobin percentage, total leucocyte count and differential leucocyte count was done using hematology analyzer. Manual differential leucocyte was also done after staining the blood smear with Leishman's stain.

Results: Final body weight of rats in Cyclosporine treated Group B was decreased significantly $p < 0.001$ in comparison to control Group A and increased significantly $p = 0.001$ in Nigella Sativa and Cyclosporine protected Group C. Blood parameters showed decreased mean hemoglobin $p = 0.03$, decreased mean total leucocyte count $p = 0.003$, increased mean neutrophil count $p = 0.27$ and decreased mean lymphocyte count $p = 0.27$ in Cyclosporine treated Group B while the mean values of these hematological parameters improved in Nigella Sativa protected Group C.

Conclusion: The present study demonstrated that Cyclosporine caused decrease in animal weight and toxic changes in hematological parameters while Nigella Sativa was helpful in ameliorating them.

Keywords: Cyclosporine, Nigella Sativa, body weight, hematology

IRB: Approved by Ethics Committee of Baqai Medical University, Ref# BMU-EC/2016-03.

Dated: 14th October 2016.

Citation: Younus N, Basharat Ali AB, Sajjad S, Mukhtar S, Rasheed A, Kazmi T. Changes in Hematological Parameters of Sprague Dawley Rats with Use of Cyclosporine and Nigella Sativa [Online]. *Annals ASH KMDC* 2020;25.

(ASH & KMDC 25(4):211;2020)

Introduction

Blood parameters like red blood corpuscles (RBC), white blood corpuscles (WBC) and hemoglobin (Hb) concentration are tightly regulated traits. They have high clinical relevance¹. Their val-

ues outside normal ranges are indicative of stressed, diseased and toxic states², and they account as the first sign of measurable toxicity of any drug^{1,2}.

Cyclosporine (CSA) is 11 amino acids containing cyclic polypeptide. It is a metabolite of *Tolypocladium inflatum*, a fungus. Its immunosuppressive properties accounts for its use in organ transplants and autoimmune diseases^{3,4,5}. It inhibit Calcineurin, which is responsible for gene transcription; in other words, it inhibits T lymphocyte signaling pathways thus preventing the activation and proliferation of T lymphocytes in target cells³.

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Date of Submission: 24th September 2020
Date of Acceptance: 17th December 2020

There are many side effects of CSA which have been attributed for its oxidative stress and reactive oxygen species productions⁴. The severity of effects is linked to doses and duration of treatment. CSA shows adverse actions on kidneys resulting in renal dysfunction and nephrotoxicity. It also shows its effects on liver leading to hepatotoxicity. Others side effects include hyperlipidemia, hypertension, hyperuricemia, hypercholesterolemia and hyperplasia of gums. Hirsutism and neurological complications like tremors are also reported. It is also known to increase total cholesterol, LDL levels and glucose levels³. In some long treated animals lymphoma and squamous cell carcinoma have also been noted⁵.

CSA also shows toxic effects in different tissues of the body. In bones it increases remodeling with appreciable bone loss while in bone marrow it inhibits the erythroid and stromal progenitors⁶. Cyclosporine has shown to increase the number of plasma cells and decrease the macrophages in humans⁶. In thymus of treated animals marked depletion of medullary region is noted⁵.

To avoid the side effects of CSA, many herbs have been reported to have positive effects. The variety of herbs include Quercetin the extract of Rhei Rhizoma, the extract of Schisandrasphenanthera. Promising effects were also seen by the use of ginger and onion⁴. This has been shown in literature that cardiotoxic effects of CSA has been reduced by the use of Nigella sativa oil in rats⁷.

Nigella Sativa (NS) (Kalonji) is a dicotyledonous seed of the Ranunculaceae family. It has been in use for over thousand years for promotion of health and as a preventive agent for various diseases⁴. It has promising immunological and antioxidant effects⁷.

The immunological effects of Nigella Sativa have been proved by different researches. NS has shown to increase the weight of lymphoid organs, increase the white blood cell production; enhance the cell mediated immune response and antibody titers⁸. Nigella Sativa oil can be used as the potential adjuvant treatment of rheumatoid arthritis as shown in literature⁴.

As far as antioxidant effects of Nigella Sativa are concerned, it has been reported to have many pharmacological and immuno-protective effects against natural and chemical toxins. Aflatoxin B1 which is a naturally occurring toxin produced by moulds in soils, hays and grains induces cell disruption which can be prevented by the use of NS⁷.

Nigella Sativa has shown to be protective against heavy metals like cadmium, aluminium and lead and chemical agents like ethanol and carbon tetrachloride. It has been used against drugs like tetracyclin, gentamycin, amikacin, bleomycin, isoniazid and acetaminophen. The toxic effects of anti-cancer drugs like cisplatin, cyclophosphamide, tamoxifen and methotrexate has been shown to be decreased by the use of NS⁷.

Nigella sativa has also been known to prevent toxicity in different organ systems. In liver, it shows the protection against antibiotics like isoniazid and oxytetracyclins and different compounds like Bisphenol A and sodium fluoride. Hepatotoxicity produced by heavy metals and chemotherapeutic agents has also been ameliorated with the use of Nigella sativa⁷.

Nephrotoxicity caused by different chemical agents and drugs can be protected by using NS. Same protective effects have been seen in heart and brain toxicities. The evidence of systemic protection of NS on reproductive and gastrointestinal systems has also been reported in different literatures.

Current literature reports that the hepatotoxicity caused by analgesics like acetaminophen, heavy metals and anti cancer drugs can be protected by the simultaneous consumption of Nigella Sativa⁷.

Present study was planned to investigate the toxicity of CSA on the blood parameters and evaluate the amelioration of NS by its immunomodulatory and antioxidant effects.

Material and Methods

This was an experimental study, designed to see the outcome of interventions. The duration of this research was 6 weeks. It was conducted in Department of Anatomy, after the research approval

from Board of Advanced Research Studies, Baqai Medical University, Karachi.

Thirty adult male albino Sprague Dawley rats were taken. These rats were 165 - 205 grams of weight and 10 - 12 weeks of age and were purchased from animal house of the university. The animals were kept in plastic cages. They were given standard rat diet ad libitum. They were acclimatized for fifteen days before commencement of study. They were taken care of according to the Ethical standards required for the animal studies as per Helsinki's resolution 1964.

NS seeds were purchased from local market, identified by pharmacist, cleaned and then used in study. CSA (Sandimmune Neorol®) was purchased from pharmacy.

All healthy albinos Sprague Dawley rats of specific age and weight were included. Any animal falling sick or dying during study was excluded from study.

After acclimatization, the animals were randomly divided into three Groups containing 10 animals each. Sample size was calculated by resource equation method. The degree of freedom of analysis of variance (E) was measured by subtracting the total number of Groups from total number of animals, which came out to be 27.

Group A was control Group, at diet ad libitum and was given no intervention. Group B, the CSA group (treatment group) received CSA oral solution at the dose of 15 mg/kg/day in single daily dose, for 21 days, through gastric gavage. Group C the NS and CSA Group (protected group) received NS whole seeds, at a dose of 450 mg/kg as one week pretreatment and simultaneously for 21 days with oral CSA solution at the dose of 15 mg/kg/day in single daily dose, for 21 days, through gastric gavage.

At the beginning of the study all animals were observed for signs of health then divided in 3 Groups. They were weighed at the start of study, during the study and at the end of the study. After the study period the animals were anesthetized and

blood samples were collected by cardiac puncture with 5cc sterile syringes. Blood was stored in EDTA tubes for peripheral smear and CBC estimation.

Blood was run through Hematology Analyzer (model: Sysmex kx-21) for complete blood count. Hematology Analyzer is a machine which uses three physical techniques for blood element detection. First is the electrical Impedance in which blood passes through a narrow aperture between two electrodes allowing only one cell to pass at a time. Each cell has its own impedance. The change in impedance is recorded thus cell count and volume of RBC, WBC and platelets is measured. Second is the flow cytometry and third is fluorescence. Reagents are added for detailed morphology of blood cells thus increasing the specificity of the machine.

Leishman's stain was used for differential lymphocyte count. Leishman's stain is a neutral stain for blood smear. It is a mixture of an acidic stain Eosin and basic stain Methylene blue diluted with buffer. It is not only used for differential leucocyte count but platelet count, toxic granules and type of anemia. The stained slides were observed under microscope and different lymphocytes were counted manually (Fig 1).

Data was analyzed for means by using SPSS (Statistical Package for Social Sciences) version 21. One way ANOVA (Analysis Of Variance) with post hoc Tuckey tests were performed to compare means between different groups, with P value of 0.05 considered significant at 95% confidence interval.

Results

The mean final body weight of the animals in control Group A was increased significantly $P < 0.001$ over the study period of 3 weeks Table 1. Final body weight of CSA treated Group B was decreased significantly from its initial body weight ($P = 0.001$) Table 1. Body weight of CSA treated Group B showed significant decrease $P < 0.001$ from final weight of control Group A Table 1 & 3. Final body weight of NS protected Group C showed insignifi-

cant $P=0.082$ increase in final body weight from initial body weight Table 1. Although final body weight of NS protected Group C showed significant increase from control Group A $P=0.006$ and from CSA treated Group B $P=0.001$ Table 1 & 3.

The mean hemoglobin (Hb) in control Group A was found to be 12.80 ± 0.16 gm/dl. It was decreased significantly in CSA treated Group B $P=0.03$ and was increased significantly in NS protected Group C $P=0.02$ Table 2 & 3.

The mean Hb in NS protected Group C was increased in comparison to CSA treated Group B but was insignificant $P=0.98$ Table 2 & 3.

The mean TLC in control Group A was found to be 6.46 ± 0.56 ($103/\mu\text{L}$). It was decreased significantly in CSA treated Group B $P=0.003$ but was decreased insignificantly in NS protected Group C $P=0.22$ Table 2 & 3.

When NS protected Group C was compared with CSA treated Group B the mean TLC was found to be increased but was insignificant $P=0.15$ Table 2 & 3.

The mean neutrophil count in control Group A was found to be 15.50 ± 0.74 %. It was increased insignificantly in CSA treated Group B $P=0.27$ but was increased significantly in NS protected Group C $P=0.001$ Table 2 & 3.

Mean neutrophil count in NS protected Group C significantly increased when compared with CSA treated Group B $P=0.04$ Table 2 & 3.

The mean lymphocyte count in control Group A was found to be 80.50 ± 0.86 %. It was decreased insignificantly in CSA treated Group B $P=0.27$ and decreased significantly in NS protected Group C $P=0.016$ Table 2 & 3.

NS protected Group C showed insignificant decreased in comparison to CSA treated Group B $P=0.35$ Table 2 & 3.

Table 1. Initial & Final Body weights of different Groups

Experimental Groups	Initial body weight (gm)	Final body weight (gm)
Group A		
No Treatment	184.50 ± 4.61	$195.30 \pm 3.97^*$
Group B		
CSA	183.80 ± 4.14	$172.60 \pm 3.87^*$
Group C		
NS + CSA	185.63 ± 3.65	186.25 ± 3.58

Mean \pm SEM (Standard Error of Mean)

*Significant difference from initial to final

Table 2. Mean Hb, TLC, Neutrophils & Lymphocyte counts of Different Groups

Groups	Treatment Given	Hb*(gm/dl) ($103/\mu\text{L}$)	TLC * count*(%)	Neutrophil count*(%)	Lymphocyte
A	No	12.80 ± 0.16	6.46 ± 0.56	15.50 ± 0.74	80.50 ± 0.86
	Treatment				
B	CSA	12.06 ± 0.26	3.91 ± 0.53	20.50 ± 0.92	75.50 ± 0.82
	(n=20)				
C	NS+CSA	12.84 ± 0.15	5.27 ± 0.37	28.60 ± 3.71	71.10 ± 3.68
	(n=20)				

* Mean \pm SEM (Standard Error of Mean)

Table 3. P value of variables in comparison to different Groups

Variables	P Value in different comparison Groups		
	A to B	A to C	B to C
Final body weight	0.001*	0.006*	0.001*
Hb	0.03*	0.02*	0.98
TLC	0.003*	0.22	0.15
Neutrophil Count	0.27	0.001*	0.04*
Lymphocyte Count	0.27	0.016*	0.016*

*Significant calculated by one way ANOVA followed by post hoc tukey's test

Discussion

The observations and results of this study showed the toxic effects of CSA and the protective effects of NS seeds on body weights and hematological parameters.

Sprague Dawley rats were used in this study. The rodents are believed to be very similar to human genome and their physiologic and metabolic processes resembles with those of humans⁹.

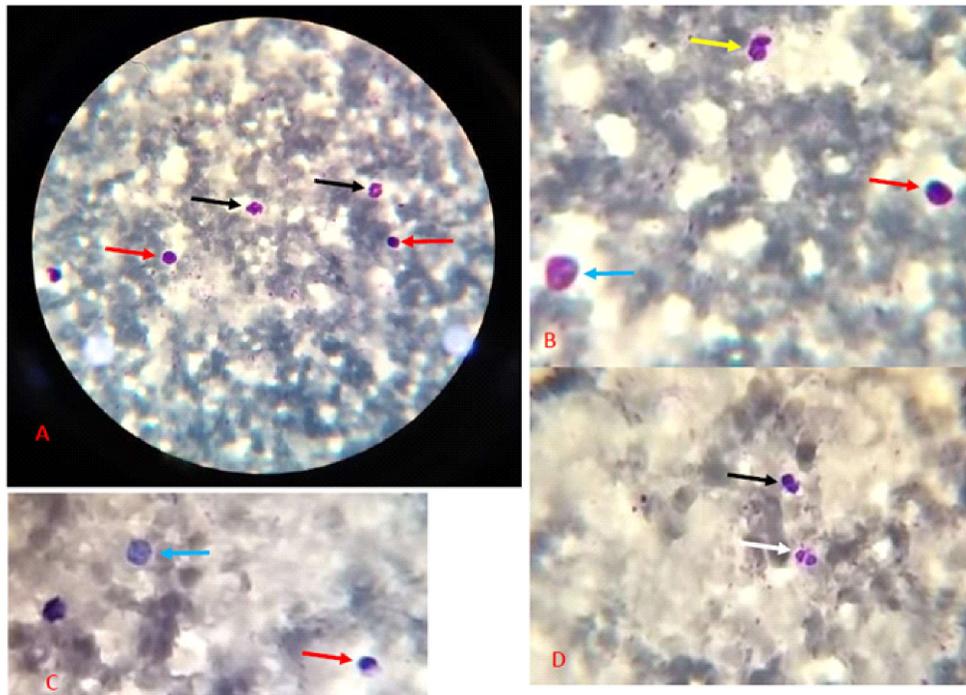


Fig 1. Leishman's stain photomicrograph showing lymphocyte (red arrow), neutrophil (black arrow), monocyte (yellow arrow), basophils, (blue arrow), eosinophil (white arrow) X 1000

Final body weights of animals in control Groups increased at diet ad libitum. This is for the fact that the normal intake of food leads to increase in body weight of rats. This fact was highlighted by the research conducted by Olsen MK et al. in which they restricted the food intake for day time only and found decrease in body weights of juvenile and adult rats. They concluded that there is a positive correlation between calorie intake and the body weight in rats¹⁰.

In our study, final body weight of animals was reduced after taking CSA in this study, same as observed by Jiang et al¹¹, the suggested mechanisms were the attenuation of animals from taking food and down regulation of gluconeogenesis by CSA thus causing lost inbody weight¹¹. Though no effect on body weights of dogs were observed by Legrand et al when administered with CSA¹².

The body weight of animals in NS protected Group C was found to be increased. This finding was also supported by the research conducted by Mosbah et al. in which they reinforced on the increase in body weight as well as organ weights in rats when given NS¹³. Positive effects of NS on body weight has also been reported in different ani-

mals. Shewita et al. studied the same in boiler chicks. They believed that NS intake increased body weight directly as well as it increased food intake of animals⁸.

Another fact showing the effect of NS on body weight was explained by Jenooobi et al. in their study. They stated that NS affects the pharmacokinetics of CSA by decreasing the oral bio availability of CSA and by increasing its clearance at its absorption site⁴.

Blood parameters are important targets for any chemical exposure to body and minute changes can predict toxicity as the earliest clinical sign^{1,2,14}.

Hb in CSA treated Group decreased this is the same as seen by Ebaid et al¹⁵. and Alenzi et al¹⁶. where different drugs and compounds decreased Hb. Elsayed et al¹⁷. showed that 4 weeks treatment with CSA causes significant decrease in Hb concentration mechanism of which is related to inhibition of erythropoietin hormone by CSA; this effect was also observed on culture medium of human Hep3 B cell line with addition of CSA¹⁷. Eldien et al. had also seen bone marrow suppression in form of degenerating cells among hematopoietic cells after CSA treatment in rats¹⁸. The decrease could be

due to decreased hematopoiesis, increased destruction of RBCs or slower rate of production as seen with CCl₄ induced haematotoxicity¹⁹. This effect is also attributed to production of free radicals by CSA¹⁹. While increased in Hb concentration in NS protected Group was also the same seen by Alenzi et al¹⁶. and Ebaid et al¹⁵. These effects were explained by anti-oxidative mechanisms triggered by NS thus reducing reactive oxygen species radicals especially if given as pretreatment¹⁶. This improvement in Hb concentration can also be explained by deductions made by Ebaid et al. that NS accelerated the cellular respiratory mechanisms needed for heme biosynthesis, the most important component in erythropoiesis¹⁵. Ajao et al. also observed increased in Hb concentration with use of NS²⁰. Though Alenzi et al. found no difference in Hb concentrations among control, NS oil and thymoquinone treated Groups¹⁶.

Total leukocyte count (TLC) and circulating lymphocytes decreased in CSA treated group also seen by Gabal et al¹⁴. and Essawy et al¹⁹. in CCl₄ toxicity. Elsayed et al. showed that CSA induced a decrease in White blood cells counts and lymphocytes due to bone marrow suppression causing leukopenia¹⁸. This is in contrast to the findings of Ebaid et al. who observed increase in TLC with chloramphenicol toxicity¹⁵.

On the other hand, Neutrophil count increased in CSA treated Group B same as seen by Elsayed et al¹⁷. with CSA treatment and Gabal et al. with CCl₄ treatment¹⁴. They both observed the same neutrophilia with anemia and leucopenia. Our result is in contrast to neutropenia found by Essawy et al. in mice due to CCl₄ toxicity¹⁹. Omar et al. also observed suppressive effects of CSA degenerative changes in neutrophils leading to neutropenia when treated with CSA⁵. While Ajao et al. observed no significant change in neutrophil and lymphocyte count²⁰, and also no effect was found on red blood indices, TLC or lymphocytes in dogs when treated with CSA¹².

NS found effective in increasing TLC in protected Group of Fararh et al²¹. seen in streptozotocin induced diabetic hamsters. TLC was also increased by use of NS oil in gamma irradiated rats²². Immuno-protective activity against CSA's immuno suppression was also studied by Vijay kumar et al using septin as an immuno modulator, where same effects were observed, like increased in TLC²³. Ajao et al. also observed im-

provement in toxic effects of Dichloros with NS oil but the toxic effects were reversed, they observed an increased in TLC²⁰.

Conclusion

This study showed that toxic changes of CSA on hematological parameters could be ameliorated with the simultaneous use of NS. CSA's clinical efficacy was affected or not, could not be deduced by above findings. For which either in vivo animal studies with introduction of disease or clinical trials in human subjects is needed. If these results can be replicated in humans than CSA's use could become safer with simultaneous use of NS.

Conflict of Interests

Authors have no conflict of interests and received no grant/funding from any organization.

References

1. Kelada SN, Aylor DL, Peck BC, Ryan JF, Tavarez U, Buus RJ et al. Genetic analysis of hematological parameters in incipient lines of the collaborative cross. *G3-Genes Genom Genet* 2012; 2:157-65. [doi: 10.1534/g3.111.001776]
2. Ololade IA, Oginni O. Toxic stress and hematological effects of nickel on African catfish, *Clarias gariepinus*, fingerlings [Online]. *J Environ Chem Ecotoxicol* 2010;2:014-9. Available from: https://academicjournals.org/article/article1379429947_Ololade%20%20and%20Oginni.pdf. Accessed on: 10th December 2020.
3. Patil U, Jaydeokar A, Bandawane D. Immunomodulators: A pharmacological review[Online]. *Int J Pharm Pharm Sci* 2012;4:30-6. Available from: <https://innovareacademics.in/journal/ijpps/Vol4Suppl1/3196.pdf>. Accessed on: 10th December 2020.
4. Al-Jenoobi F, Al-Suwayeh S, Muzaffar I, Alam MA, Al-Kharfy KM, Korashy HM, et al. Effects of NS and *Lepidium sativum* on CSA pharmacokinetics [Online]. *Biomed Res Int* 2013; 2013:953520. Available from: <https://www.hindawi.com/journals/bmri/2013/953520/>. Accessed on: 10th December 2020
5. Omar HE-DM, Eldien HMS, Badary MS, Al-Khatib BY, AbdElgaffar SK. The immunomodulating and antioxidant activity of fucoidan on the splenic tissue of rats treated with cyclosporine A. *J Basic Appl Zool* 2013; 66:243-54. Available from: The immunomodulating and antioxidant activity of fucoidan on the splenic tissue of rats treated with CSA A. Accessed on: 10th December 2020.
6. Eldien HMS, Omar HE-DM, Badary MS, Al-Khatib BY. The possible protective and therapeutic roles of fucoidan in CSA-induced histological changes in the bone marrow and spleen in rats [Online]. *Egypt J Histol* 2012; 35:383-97. Available from:

- https://journals.lww.com/ejhistology/Fulltext/2012/09000/The_possible_protective_and_therapeutic_roles_of.3.aspx. Accessed on: 10th December 2020.
7. Tavakkoli A, Ahmadi A, Razavi BM, Hosseinzadeh H. Black seed (NS) and its constituent thymoquinone as an antidote or a protective agent against natural or chemical toxicities [Online]. *Iran J Pharm Res* 2017; 16:2. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5963642/>. Accessed on: 10th December 2020.
 8. Shewita R, Taha A. Effect of dietary supplementation of different levels of black seed (NS L.) on growth performance, immunological, hematological and carcass parameters of broiler chicks. *World Acad Sci Eng Technol* 2011; 77:788-94. Available from: <https://zenodo.org/record/1072271#.X9MNdOaSnIU>. Accessed on: 10th December 2020.
 9. Sengupta P. The laboratory rat: relating its age with human's. *Int J Prev Med* 2013;4:624-30.
 10. Olsen MK, Choi MH, Kulseng B, Zhao CM, Chen D. Time-restricted feeding on weekdays restricts weight gain: A study using rat models of high-fat diet-induced obesity. *Physiol Behav* 2017; 173:298-304. [doi: 10.1016/j.physbeh.2017.02.032.]
 11. Jiang M, Wang C, Meng Q, Li F, Li K, Luo L, et al. Cyclosporin A attenuates weight gain and improves glucose tolerance in diet-induced obese mice. *Mol Cell Endocrinol* 2013;370:96-102. [doi: 10.1016/j.mce.2013.02.018]
 12. Legrand JJ, Bouchez C, Mimouni C, N'Guyen A, Bouchard J, Ameller T, et al. Immunotoxic effects of cyclophosphamide and CSA in the dog [Online]. *J Immunotoxicol* 2013;10:90-5. Available from: <https://www.tandfonline.com/doi/full/10.3109/1547691X.2012.723766>. Accessed on: 10th December 2020
 13. Mosbah R, Djerrou Z, Mantovani A. Protective effect of Nigella sativa oil against acetamiprid induced reproductive toxicity in male rats. *Drug Chem Toxicol* 2018;41:206-12. [doi: 10.1080/01480545.2017.1337127]
 14. Gabal AA, Essawy A, Abdel-Moneim A, Hamed S, Elzergy A. The protective effect of black seed (NS) against carbon tetrachloride-induced chromosomal aberrations and ultrastructural changes of bone marrow cells [Online]. *Arab J Biotechnol* 2007;10:275-88. Available from: https://www.researchgate.net/publication/235326932_The_protective_effect_of_black_seed_Nigella_sativa_against_carbon_tetrachloride-induced_chromosomal_aberrations_and_ultrastructural_changes_of_bone_marrow_cells. Accessed on: 10th December 2020.
 15. Ebaid H, Dkhil MA, Zahran WS, El Feki MA, Gabry MS. Role of NS in ameliorating chloramphenicol induced tissue damage in rats [Online]. *J Med Plant Res* 2011;5:208-88. Available from: https://www.researchgate.net/publication/228489199_Role_of_Nigella_sativa_in_ameliorating_chloramphenicol_induced_tissue_damage_in_rats. Accessed on: 10th December 2020.
 16. Alenzi F, El-Bolkiny YE-S, Salem M. Protective effects of NS oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. *Br J Biomed Sci* 2010;67:20. [doi: 10.1080/09674845.2010.11730285]
 17. Elsayed AS, Jbireal JM, Azab AE. Effect of acute and chronic CSA A treatment on hematological data in male albino rats [Online]. *J Appl Biotechnol Bioeng* 2018;5:350-7. Available from: <https://medcraveonline.com/JABB/effect-of-acute-and-chronic-cyclosporine-a-treatment-on-hematological-data-in-male-albino-rats.html>. Accessed on: 10th December 2020.
 18. Eldien HM, Omar HE, Badary MS, Al-Khatib BY. The possible protective and therapeutic roles of fucoidan in cyclosporine-induced histological changes in the bone marrow and spleen in rats. *Egyptian Journal of Histology* 2012;35:383-97. Available from: https://www.researchgate.net/publication/271734300_The_possible_protective_and_therapeutic_roles_of_fucoidan_in_cyclosporine-induced_histological_changes_in_the_bone_marrow_and_spleen_in_rats. Accessed on: 10th December 2020.
 19. Essawy AE, Hamed SS, Abdel-Moneim AM, Abou-Gabal AA, Alzergy AA. Role of black seeds (NS) in ameliorating carbon tetrachloride induced haematotoxicity in Swiss albino mice [Online]. *J Med Plant Res* 2010;4:1977-86. Available from: <https://academicjournals.org/journal/JMPR/article-abstract/8F0E8ED15726>. Accessed on: 10th December 2020.
 20. Ajao MS, Sansa AB, Imam A, Ibrahim A, Adana MY, Alli-Oluwafuyi A, et al. Protective Effect of NS (Black Caraway) oil on oral dichlorvos induced hematological, renal and nonspecific immune system toxicity in Wistar rats. *Iran J Toxicol* 2017;11:1-5. Available from: https://www.researchgate.net/publication/320946322_Protective_Effect_of_Nigella_Sativa_Black_Caraway_Oil_on_Oral_Dichlorvos_Induced_Hematological_Renal_and_Nonspecific_Immune_System_Toxicity_in_Wistar_Rats. Accessed on: 10th December 2020.
 21. Fararh K, Atoji Y, Shimizu Y, Shiina T, Nikami H, Takewaki T. Mechanisms of the hypoglycaemic and immunopotentiating effects of NS L. oil in streptozotocin-induced diabetic hamsters. *Res Vet Sci* 2004;77:123-9. [doi: 10.1016/j.rvsc.2004.03.002.]
 22. Assayed ME. Radioprotective effects of black seed (NS) oil against hemopoietic damage and immunosuppression in gamma-irradiated rats. *Immunopharm Immunot* 2010;32:284-96. [doi: 10.3109/08923970903307552.]
 23. Vijaykumar N. Evaluation of Immunoprotective Activity of Septilin against CSA A induced Immune Suppression [Online]. *Curr Res Pharm Sci* 2016;6:55-62. Available from: <https://www.semanticscholar.org/paper/Evaluation-of-Immunoprotective-Activity-of-Septilin-Vijaykumar/071c51ab9704462ad2ea5e620f4156421be8cd0e>. Accessed on: 10th December 2020.