Active-site Molecular docking of Nigellidine to nucleocapsid/Nsp2/Nsp3/MPro of COVID-19 and to human IL1R and TNFR1/2 may stop viral-growth/cytokine-flood, and the drug source Nigella sativa (black cumin) seeds show potent antioxidant role in experimental rats.

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Abstract

The recent outbreak of SARS CoV-2 has changed the global scenario of human lives and economy. In this pandemic-outbreak the ratio of infected person is much higher than the death encountered. Most of the dead patients were observed with dysfunction/failure of cardiac and renal systems. Beside this a 'cytokine storm' namely TNF-α/IL1 receptors i.e. TNFR1/TNFR2/IL1R over-functioning was reported in the infected-persons. Here, nigellidine, an indazole-alkaloid and key-component of *Nigella Sativa L.* (NS); black-cumin-seed, has been analyzed for COVID-19 different protein and TNFα receptors TNFR1/TNFR2 and IL1R inhibition through molecular-docking study and biochemical-study of cumin-seed extract exposure to experimental-rat. The NMR, X-ray-crystallographic or Electron-microscopic structures of COVID-19 Main-protease(6LU7), Spike-glycoprotein(6vsb), NSP2(QHD43415_2), N-terminus-protenase (QHD43415_3), Nucleocapsid(QHD43423) and Human IL1R (1itb), TNFR1 (1ncf), TNFR2 (3alq) from PDB were retrieved/analyzed for receptor-ligand interaction in normal condition. Then those structures were docked with nigellidine using Autodock-software and Patchdock-server. Where nigellidine showed highest binding-energy of -7.61 (kcal/mol) and ligand-efficiency value of (-0.35) forming bonds with amino acids THR943/LYS945/MET1556/ALA1557/PRO1558/ILE1559. Highest ACE-value of -356.72 was also observed for nigellidine N-terminal-protease interaction. Nigellidine also showed strong interaction with NSP2 (-6.28) and Mpro/3CLpro_Q (-6.38s). Nigellidine showed affinity to TNFR1 (-6.81), IL1R (-6.23) and TNFR2 (-5.16). In rat experiment 2-groups (vehicle and NS treated) of female Wistar-rats were taken for experiments. The NS treated tissue showed marked decline in ALP/SGPT/ SGOT/MDA level then the basal-levels. From the Western-blot or activity analysis it was observed that Nigellidine, the sulfuryl-group containing drug showed no impact on Phenol-catalyzing ASTIV or Steroid-catalyzing EST expressions/activities and thus have no influence in sulfation-mediated adverse metabolic-processes. Current-results concluded that Nigellidine has hepato/reno-protective; immunomodulatory/anti-inflammatory and antioxidant activities as well as it inhibits important proteins of COVID-19. With steps to further validation/checking nigellidine can be used in COVID-19 infection.

Introduction

The pandemic outbreak from SARS CoV 2 has claimed a large number of lives from millions of infections globally. A horrified and lockdown situation has generated a post infection and post remedial traumatized situation that is creating a long term health hazards also. When the number of deaths alone is counted it is found to be no doubt huge but when it is calculated with respect to the number of infections it is found approximately one of twentieth. The question arise how these nineteen people get escape or what type of physiological measures they possess. In other words, what condition makes that one unfortunate person to be more morbid? Is it not the point that co-morbid status might have increased the risk of death? When it is noticed from the statistics that elder persons and males are more affected then it can be clarified that these two factors can increase the risk. One of the adverse physiological conditions in the elder persons is their weaker immunological status than the younger one. Nevertheless, overflow of the inflammatory

responses make the situations more unfavorable in these patients. In this conditions specific new drug targeting, drug repurposing for the specific proteins on the CoV 2 structure and vaccination strategies are the demand of the time. Our laboratory is screening from a large number of drug target for the purpose. Some traditional molecules as phytochemical sources have been tested here and found that nigellidine from *Nigella Sativa L.*; black cumin seed may be of great importance.

It (Ranunculaceae) is an annual herb extensively used in the Middle-East, India and other countries. Its traditional use is reported in ancient texts and historical documents [1]. The first indazole alkaloids nigellidine and nigellicine which can remain in sulfated-form are accumulated in this seed coat B. Sulfated form increases its polarity and bioavailability. Nigellicine, nigellidine, thymoquinone, dithymoquinone from this extract has shown strong as anti-oxidant, anti-bacterial, anti-hypertensive, anti-inflammatory and immunomodulatory effects [2,3].Toxicity studies from our lab and other have suggested the safety of N. sativa extract in different form i.e. fat soluble or water soluble [4].

Antioxidant role of NS extract has been shown by lower serum levels of MDA in the experimental rats suggest that it can be used in oxidative stress condition. No significant impact was found on TNF-α, IL-10 and hs-CRP levels of the exposed group [5]. But the eventual inflammatory signaling has not been tested here. Or their receptor binding capacity has not been screened. Nigellidine has been shown to be immunomodulatory and anti-inflammatory. Beside immunological protection, NS serves as metabolic protector.

Administration of *Nigella sativa* might improve lipid and blood sugar profile in postmenopausal women with different types of metabolic syndrome [6,7], and it also act as hepato- and reno-protective compound [8]. NS seed proteins has potential as therapeutic agent for cancer [9]. Most importantly, in SARS CoV-2 infection ACE-2 mediated impairment of aldosterone system may be repaired by NS. Vasorelaxant and anti-hypertensive function of NS helps in the modulation of renin angiotensin system (RAS) or the diuretic activity [10], which is one of the major targets of COVID. It might have great protective role during post infective secondary disorder of the peripheral vasculature namely cardiac and renal systems. In most of the instances patients die due to this organ dysfunction/failure in COVID-19 infection.

Nigella sativa was shown to significantly improve laboratory parameters of hyperglycemia and diabetes control, glycated hemoglobin, and insulin resistance, and a rise in serum insulin [7].

In this background, present study tested the possible active site docking of nigellidine to several important SARS CoV 2 proteins and the human inflammatory molecules which are reported to create a 'cytokine storm' namely TNF-α receptors i.e. TNFR1, TNFR2 and IL1R. In the experimental rat model the source of this drug *Nigella sativa*; black cumin seed extracts were tested for its role on antioxidant, hepatic and renal status. This work will help in the urgent therapeutic intervention against COVID-19 global pandemic.

Materials And Methods

Bioinformatics and Molecular docking experiment

Protein Structure Retrieval

The NMR, electron microscopy and X-ray crystallography structures were retrieved from RCSB PDB (https://www.rcsb.org/) and Zhong Lab (https://zhanglab.ccmb.med.umich.edu/COVID-19/). Structure of Main protease (6LU7), Spike glycoprotein (6vsb) of human coronavirus-2 and different human receptors and other proteins like IL1R (1itb), TNFR1 (1ncf), TNFR2 (3alq) were retrieved from PDB. Whereas, NSP2 (QHD43415_2), N-terminus protenase (QHD43415_3), Nucleocapsid (QHD43423) and C-terminus protease (QHD43415_5) from coronavirus were retrieved from Zhang Lab. Maximum structures were observed to bind with their respective ligands.

Ligand Structure Retrieval

The three dimensional (3D) structures of nigellidine were retrieved in .sdf format from world's largest chemical information database, PubChem (https://pubchem.ncbi.nlm.nih.gov/). The PubChem CID, canonical SMILES, molecular weight, molecular formula were 136828302 and CC1=CC(=O)C2=C(N3CCCCN3C2=C1)C4=CC=C(C=C4)O, 294.3 g/mol and $C_{18}H_{18}N_2O_2$.

Preparation of both Receptor and Ligand Molecules

Receptor molecules both from human coronavirus and human receptor and other molecules were found with numerous water molecules, ions and in some their respective ligands. The water and ions were removed and rest of the structures was saved in .pdb format. For ligand bound receptors initially the interaction patterns were analyzed using Pymol molecular graphics visualize and documented. Then the ligand molecules were removed and receptor molecules were prepared for molecular docking with nigellidine and the modified files were saved in .pdb format. The .sdf format of chemical ligand was converted to .pdb also using Pymol.

Molecular Docking

The molecular docking of nigellidine with all the selected proteins was performed through AutoDock 4.2 [11] offline software and Patchdock online server for interactive docking. Autodock calculates the global minimum energy in the interaction between the substrate and the target protein, calculating all the degrees of freedom (DOF) available for the system. During docking parameter file generation, all nonpolar hydrogens were removed except polar and charged one. The receptors were added with Kolman Charges and ligand was calculated for Gasteiger charges and saved in PDBQT format then these formats were used for molecular docking. PatchDock is developed as geometry-based molecular docking algorithm. It calculates the docking transformation between two molecules to get the best molecular interface complementarity which finds out the ligand posture in receptor with maximum interface area covered and minimum steric hindrance [12]. It also calculates the Atomic Contact Energy (ACE) Value of each docking positions to indicate the amount of required desolvation free energies to transfer the ligand molecule

from water to protein (Receptor) interior. The docked structures were visualized and represented using Pymol and AutoDock tool. The drug-protein interactions were represented through LIGPLOT software.

Biochemical impact of black-cumin seed-extract exposure to experimental rat

Female Wistar rats were purchased from a small-animal firm house (govt. registered) that follows all ethical norms and maintain requisite regulatory affairs. The firm house is a Government accredited (CPCSEA-Committee for the Purpose of Control and Supervision of Experiments on Animals: Reg. no 1A2A/PO/BT/S/15/CPCSEA. < http://cpcsea.nic.in/Auth/index.aspx >) organization under the Dept. of Animal Husbandry and Dairy, Ministry of Agriculture and Farmer's Welfare, Govt. of India. For all animal experiments, proper permissions were obtained from the Institutional (Oriental Institute of Science and Technology) Review Board.

Rats of ageing 3 to 4 weeks were acclimatized for 10 days at 12-hour light-dark cycle, 25ºC ± 2ºC temperature, 50%–70% humidity in the institutional animal resource facility. Those were fed with a standard pellet diet (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. Studies were carried out in accordance with the National Institutes of Health, USA guidelines and the institutional ethical concerns were maintained throughout the investigation. Rats were randomly distributed in 2 groups (vehicle and NS treated) having 6 in each.

On the day of sacrifice, animals were experienced cervical dislocation and initially their blood was collected using a disposable syringe (21-gauge needle), serum was separated from the collected blood samples. The liver tissue was carefully collected and stored at -20ºC for experimental purposes.

Evaluation of General Toxicity.

Serum glutamate pyruvate transaminase (SGPT), SGOT, alkaline phosphatase (ALP), urea, and creatinine were measured from the rats by standard protocol with the assay kits (Ranbaxy, India or other reputed company).

Estimation of Malondialdehyde (MDA) Levelsin liver tissue

Liver tissue was homogenized (10 % w/v) in the ice-cold phosphate buffer (0.1 mol /L, pH 7.4) and the homogenate was centrifuged at 10,000 rpm at 4ºC for 10 min. The MDA assay was conducted using the supernatant following the protocol of Buege and Aust, 1978 [13,14]. To chelate iron and reduce its interference in peroxidation reaction of unsaturated fatty acid, 1 mM EDTA was used in the reaction mixture. To reduce the interference caused by a yellow-orange colour produced by some carbohydrates, the reaction mixture was heated at 80oC instead of 100ºC. Finally, the MDA was   measured and calculated utilizing the molar extinction coefficient of MDA (1.56×10^5 cm2/ mmol).

Estimation of Non Protein Soluble Thiol (NPSH) in liver tissue

The NPSH in serum and liver tissue homogenates (prepared in 0.1 M phosphate buffer, pH 7.4) were determined by the standard DTNB (5, 5'- dithiobis-2-nitrobenzoic acid) method with a slight modification [26]. In brief, the protein was precipitated by trichloroacetic acid and clear cytosol was added to 0.1 M sodium phosphate buffer containing 5 µM DTNB. The level of NPSH was determined against a GSH standard curve [15].

ASTIV activity (PNPS assay method) in liver tissue

β-Naphthol sulfation activity from liver cytosols was determined as previously described [16,17]. This assay determines phenol sulfation activities of different isoforms of phenol sulfating SULTs. Briefly, sulfation activity was determined in a reaction mixture containing 50mM Tris buffer, pH 6.2, 5mM PNPS, 20 µM PAPS, and 0.1mM β-naphthol. Rat liver cytosols (50 µg protein) were used as the enzyme source in a total reaction volume of 250 µl. After 30 min incubation at 37°C in a shaking water bath, the reaction was stopped by adding 250µl of 0.25M Tris, pH 8.7. The reaction mixtures were read at 401 nm in a spectrophotometer. Specific activity (SA) was expressed as nanomoles per minute per milligram of protein. The data shown in the figures are the average of data sets collected from 6 different animals.

Western blot analysis of Estrogen sulfotransferase (SULT1E1) in liver tissue

Western blot was conducted following an earlier standardized protocol [18] as in Maiti et al. 2007 with a slight modification. A 12 % denaturing gel was loaded with 25 µg of protein and electrophoresis was done at 100v for 3 hours, transfer was done at 100v for 2 hours. The membrane was washed and incubated in primary and secondary antibodies as mentioned in the protocol. Brown coloured bands were developed by using Diaminobenzidine (DAB).

Results And Discussion

Viral protein used in this experiment; https://zhanglab.ccmb.med.umich.edu/COVID-19/

Nigellidine binding to N terminus protease nsp3_QHD43415_3 (Fig 1)

This is known as Papain-like proteinase. It is responsible for the cleavages located at the N-terminus of the replicase polyprotein. It participates together with nsp4 in the assembly of virally-induced cytoplasmic double-membrane vesicles necessary for viral replication. Nigellidine showed highest binding energy value of -7.61 and its ligand efficiency was also high (-0.35). According to the AutoDock result the position A having binding energy -7.61, forms some unstable bonds with amino acids THR943, LYS945, MET 1556, ALA1557, PRO1558, ILE1559 (Fig 1). Binding energy range of -7.59 to -4.56 (total posture 4) were found at position B. It showed an entry/exit pocket where nigellidine binds to TRP1632 through an N-O bond and other hydrogen bond with HIS1630, TRP1632, ALA1878 and THR1774. The ACE value was also good at this site (-284.28) and nigellidine affinity was also found. At site C binding energy (-6.18 & -5.05) was also high and the highest ACE value (-356.72) was observed. Here also nigellidine formed stable bond with GLY251 (C-C & O-C) and

ASN 244 (N-O) and some hydrogen bond with other mentioned amino acids. From this observation it could be concluded that affinity of nigellidine at site was very high but then it may transferred to site B and C and may hamper the normal function of N-terminal protease of coronavirus.

Nigellidine binding to Proteinase 3CL-PRO or Mpro_QHD43415_5 (Fig 2).

This protein cleaves the C-terminus of replicase polyprotein at 11 sites. Recognizes substrates containing the core sequence [ILMVF]-Q-|-[SGACN]. Also able to bind an ADP-ribose-1"-phosphate (ADRP). It is noticed from both Patchdock and Autodock results nigellide binds with the high efficiency with the important amino acids in the active sites of the enzyme which could have altered its activity and viral metabolism significantly. The ACE value was found to be -265. 82. Active site biding energy was found to be -6.38 and -4.19 suggesting potential impairment due to drug binding effect. One previous report suggests Mpro protease structure in COVID-19 may bind nigellide molecule at it active site [19].

Nigellidine binding to Non-structural protein 2 nsp2_QHD43415_2; *Fig 3*

This protein plays a role in the modulation of host cell survival signaling pathway by interacting with host PHB and PHB2, which helps to maintain the functional integrity of the mitochondria and protecting cells from various stresses. According to Angeletti et.al., 2020 [20], NSP2 protein of COVID 19 has an entry pocket (Fig 3). Just at that position nigellidine bound with ACE value of -248.81 and -241.81 forming an H-bond with ALA 241. Same location with binding energy value of -6.28 (out of the pocket) and -5.86 (within the pocket) was observed in AutoDock result. The ligand efficiency was found −e site also.

Nigellidine binding to Nucleocapsid QHD43423 −*Fig 4*

This is one of the important proteins that packages the positive strand viral genome RNA into a helical ribonucleocapsid (RNP) and plays a fundamental role during virion assembly by interacting viral genome and membrane protein M. It also plays an important role in enhancing the efficiency of subgenomic viral RNA transcription as well as viral replication. According to Luo et al., 2004, [21] nucleocapside of COVID − 19 has a conserved sequence of WPQIAQF which acts as the active site. Nigellidine was bound with the 6th Q residue stably in docking experiment. So, it could directly inhibit the activity of viral nucleocapsid. The highest binding energy value was -6.24 and the active site binding energy was -4.9. And the ligand efficiency values were -0.28 and -0.22.

Nigellidine binding to Spike Glycoprotein, data not shown

The spike glycoprotein interact with the angiotensin convertage enzyme 2 (ACE 2) receptor present at human cell surface and initiate the viral entry into the host cell. No satisfactory attachment of nigellidine

was observed at the active site. The highest binding energy value was -6.11, whereas, presence of nigellidien at the central core was observed with -4.77 binding energy. The ligand efficiency ranged from -0.28 to -0.22. Though the highest ACE value was -340.50 and strong affinity was observed .

Human receptors, inflammatory signal molecules and other proteins

Nigellidine binding to IL1R, Fig 5

The highest binding energy for IL1R and nigellidine was -6.23 but that location was not at the active site. IL1R interact with its ligand through the amino acids TYR 127, VAL 16, ALA 109, GLU 11, ILE 110, LYS112, LEU 237, ASP 239, ALA 241, TYR261, ASP 260 and GLU 252. Among them nigellidine was found to interact with TYR127 (ACE value: -216.30) and TYR261 (ACE value: -136.97). Additionally it was found at the location LYS270, THR300 and HIS301 (ACE value: -64.99). This location is very close to ligand attachment site. It may also destabilize ligand interaction with receptor. At that location -5.67 and -5.23 binding energy value was also observed in Autodock result. And the ligand efficiency were -0.26 and -0.24.

Nigellidine binding to TNFR1, Fig 6

A strong affinity of nigellidine was found with TNFR1. According to Autodock, It showed a range of binding energy value -6.81 to -5.81. All the postures were found at a secondary attachment site of TNFR1. And their ligand efficiency value were also remarkable ranging from -0.31 to -0.26. Normally TNFR1 interact with its ligand involving the amino acids Met 11, SER 13, GLN 48, LYS 32, GLU 64, ASP 49, LYS 35, GLU 54, GLN 17, GLN 130, GLN 133 and THR 135. Among them, GLN 130, GLN 133 and THR 135 were present at the secondary attachment site which could be blocked by nigellidine. Similar results also analyzed in Patchdock result. Additionally some interaction was also observed at the primary binding sites according to ACE value where nigellidine was found to block GLN 17 and ASP 49. This indicated the strong TNFR1 inhibition affinity of nigellidine.

Nigellidine binding to TNFR2 Fig 7

In TNFR2 normal interaction with its ligand occurs through the amino acids ARG 113, CYS 74, SER 73, SER 59, CYS 71, TYR 103, ARE 13, GLN 109, GLN 63, TRP 67 and CYS 71. All the interactions were stabilized through hydrogen bond. Whereas, nigellidine formed H-bond with SER153, THR 151, ARG 158, ASP 175, ASN 149 and TYR61. In addition some stable bonds were also formed with GLN 63, GLN 109, PRO 144, ILE 162, GLY 145, LYS 120 and GLU 84. Most interestingly amino acids GLN 63, GLN 109 if occupied by nigellidine it will hamper the normal ligand interactions. Although the ACE value of TNFR2 – Nigellidine binding was moderate and the highest value was -258.71. But, the normal function could be

hampered if nigellidine concentration will increase. The highest binding energy value for this interaction was -5.16 and the ligand efficiency value was ranged from -0.23 −0.19.

Results on rat experimental rat model

Present results suggest in the figure 8 that ALP, SGPT and SGOT and MDA remarkably lower in the NS treated tissues compared to that of their basal level. Serum urea and creatinine levels were also found to decline from that of vehicle treated group (data not shown). Nigellidine is the first indazole alkaloid which has been found with a sulfuryl group. The sulfated compound is possibly favorable for higher rate of solubility and increased bioavailability. So, this compound might have role in the induction of sulfotranseferase; SULTs that catalyzes sulfuryl group transfer. These enzymes may modulate phase II drug metabolism, drug-drug interactions. In the current study, we found no impact of nigellidine on Phenol catalyzing ASTIV or Steroid catalyzing EST expressions/activities. So this drug does not influence at least sulfation mediated adverse metabolic processes. This is demonstrated in the figure 8.

Our present finding has two parts. In the molecular docking strategies with both PatchDock and Autodock were run to verify the binding of nigellidine and its locations on different proteins of SARS CoV 2. It is noticed that the drug very effectively binds with the N terminus protease, nucleocapsid and Main protease which are absolutely important for viral maturations in proteins structures, RNA packaging and other functions. Another point is that during post infection period this virus has been reported to greatly impair the human immune system by the extravagant activities of different cytokines namely TNFα, IL1, IL6 and others. These signaling molecules perform after binding with their specific receptors like TNFR1/2, IL1R and IL6R respectively. So blocking of these cytokines or their receptors may help to break the cascade of cytokine signaling. This could be one of the steps to decrease the severity of the SARS CoV 2 infection. Other than nigellidine there are several compounds like thymoquinone groups of drugs, α-hederin and these compounds are reported to have significant therapeutic activities against different types of pathogen infections. The docking results revealed promising inhibitory potential of thymoquinone against Cag A and Vac A, H. pylori oncoproteins with comparison to the standard drug, metronidazole [22].

Complex glycan molecules are the frequent components on the viral and bacterial surfaces; sometimes lipopolysaccharides or glycated proteins generate significant and unwanted immunological reactions which instead potentiating, exhaust the host immune system. As for example, in the present case, SARS CoV 2 spike which is covered with a large number of NAG disfavors the proper presentation of the epitopic part at the time of MHC presentations. This glycan molecule also restricts proper drug targeting to the spike proteins. This has been demonstrated in our previous findings on some suitable epitope-screening from SARS CoV 2 spike [23]. Our earlier study also demonstrated that *Epigallocatechin gallate* (EGCG) and *Theaflavin gallate* (TDG) are the potent binder to the CoV 2 spike channel [24]. Increased cytokines and other hematopoetic parameters (oxidative stress markers) by LPS-administration have been shown to terminate by Nigella sativa (NS) extract [25]. Cardio-respiratory and endothelial dysfunction is one of the major symptoms of SARS CoV 2 infection. And this dysfunction may be

counteracted by NS and its component thymoquinone by restraining interleukin-1β, TNF-α and NF-κB signaling [26]. Promising anxiolytic and anti-inflammatory activities of NS has been demonstrated [27]. Hypertensive renin-angiotensin system is an obvious co-morbid condition in the elderly persons and that has been targeted in the SARS CoV 2 infection. Nevertheless, hypertension associated diabetic immune-suppressive status is the major target of this viral infection. So drug prohibition of these secondary disorders is of great importance in decreasing mortality rate. The NS component, nigellidine has been decisively shown to bind to the active sites of the IL1 and TNF-α receptors (fig 6 and 7). The immune response and pathogenicity of the H9N2 avian influenza virus has been restricted by the Nigella sativa [28]. The use of N. sativa seed fixed oil can inhibit the inflammation of sinuses and respiratory airways, microbial infections, such as coryza, nasal congestion [29]. Report revealed from some prospective study that, killed and re-assorted influenza virus supplemented with natural adjuvant like Nigella sativa has been more responsive than NS alone to generate IgG and IgM responses via augmented CD4+- and CD8+ signaling [30]. A significant decline in the number of peripheral lymphocyte counts, mainly CD4 T and CD8 T cells in COVID-19 patients is shown to relate disease severity and further opportunistic infection. Several peripheral; tissues like spleen, lymph nodes, and lymphoid tissue and T lymphocytes have been shown to carry COVID-19 RNA. It is suggested that enhanced CD4+ mediated responses generated by NS in the decrease of HIV-RNA load in the patients. [31]

In the current study, we have decisively shown by molecular modeling that nigellidine can bind in the active sites of several important proteins of SARS CoV 2, several host receptors specific for SARS CoV 2 induced inflammatory markers IL1, IL6, TNF-α. Moreover, the extract from black cumin seed has been shown in experimental rat to be highly antioxidative, hepato- and reno-protective. Further studies are necessary to verify the potential effects of nigellidine in in vivo laboratory experimental animal model.

References

1. otnick I, Xue W, Bar E, et al. Distribution of primary and specialized metabolites in Nigella sativa seeds, a spice with vast traditional and historical uses. *Molecules*. 2012;17(9):10159–10177. Published 2012 Aug 24. doi:10.3390/molecules170910159

2. Shakeri F, Gholamnezhad Z, Mégarbane B, Rezaee R, Boskabady MH. Gastrointestinal effects of Nigella sativa and its main constituent, thymoquinone: a review. *Avicenna J Phytomed*. 2016;6(1):9–20.

3. Szerlauth A, Muráth S, Viski S, Szilagyi I. Radical scavenging activity of plant extracts from improved processing. *Heliyon*. 2019;5(11):e02763. Published 2019 Nov 14. doi:10.1016/j.heliyon.2019.e02763

4. Majdalawieh AF, Fayyad MW, Nasrallah GK. Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of Nigella sativa. *Crit Rev Food Sci Nutr*. 2017;57(18):3911–3928. doi:10.1080/10408398.2016.127797

5. Amizadeh S, Rashtchizadeh N, Khabbazi A, et al. Effect of *Nigella sativa* oil extracts on inflammatory and oxidative stress markers in Behcet's disease: A randomized, double-blind, placebo-controlled clinical trial. *Avicenna J Phytomed*. 2020;10(2):181–189.

6. Shirazi M, Khodakarami F, Feizabad E, Ghaemi M. The effects of nigella sativa on anthropometric and biochemical indices in postmenopausal women with metabolic syndrome [published online ahead of print, 2020 Mar 31]. *Endocrine*. 2020;10.1007/s12020-020-02265-w. doi:10.1007/s12020-020-02265-w

7. Hamdan A, Haji Idrus R, Mokhtar MH. Effects of *Nigella Sativa* on Type-2 Diabetes Mellitus: A Systematic Review. *Int J Environ Res Public Health*. 2019;16(24):4911. Published 2019 Dec 5. doi:10.3390/ijerph16244911

8. Razmpoosh E, Safi S, Abdollahi N, et al. The effect of Nigella sativa on the measures of liver and kidney parameters: A systematic review and meta-analysis of randomized-controlled trials [published online ahead of print, 2020 Mar 20]. *Pharmacol Res*. 2020;156:104767. doi:10.1016/j.phrs.2020.104767

9. Khurshid Y, Syed B, Simjee SU, Beg O, Ahmed A. Antiproliferative and apoptotic effects of proteins from black seeds (Nigella sativa) on human breast MCF-7 cancer cell line. *BMC Complement Med Ther*. 2020;20(1):5. Published 2020 Jan 13. doi:10.1186/s12906-019-2804-1

10. Ajebli M, Eddouks M. Phytotherapy of hypertension: An updated overview [published online ahead of print, 2019 Dec 26]. *Endocr Metab Immune Disord Drug Targets*. 2019;10.2174/1871530320666191227104648. doi:10.2174/1871530320666191227104648

11. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem*. 2009;30(16):2785-2791. doi:10.1002/jcc.21256

12. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Res*. 2005;33(Web Server issue):W363–W367. doi:10.1093/nar/gki481

13. Maia Mda S, Bicudo SD, Sicherle CC, Rodello L, Gallego IC. Lipid peroxidation and generation of hydrogen peroxide in frozen-thawed ram semen cryopreserved in extenders with antioxidants. *Anim Reprod Sci*. 2010;122(1-2):118-123. doi:10.1016/j.anireprosci.2010.08.004

14. Nazmeen A, Chen G, Ghosh TK, Maiti S. Breast cancer pathogenesis is linked to the intra-tumoral estrogen sulfotransferase (hSULT1E1) expressions regulated by cellular redox dependent Nrf-2/NF$\kappa\beta$ interplay. *Cancer Cell Int*. 2020;20:70. Published 2020 Mar 4. doi:10.1186/s12935-020-1153-y

15. Nazmeen A, Maiti S. Oxidant stress induction and signalling in xenografted (human breast cancer-tissues) plus estradiol treated or N-ethyl-N-nitrosourea treated female rats via altered estrogen sulfotransferase (rSULT1E1) expressions and SOD1/catalase regulations. *Mol Biol Rep*. 2018;45(6):2571-2584. doi:10.1007/s11033-018-4425-z

16. Maiti S, Chen G. Ethanol up-regulates phenol sulfotransferase (SULT1A1) and hydroxysteroid sulfotransferase (SULT2A1) in rat liver and intestine. *Arch Physiol Biochem*. 2015;121(2):68-74. doi:10.3109/13813455.2014.992440

17. Maiti S, Dutta SM, Baker SM, et al. In vivo and in vitro oxidative regulation of rat aryl sulfotransferase IV (AST IV). *J Biochem Mol Toxicol*. 2005;19(2):109-118. doi:10.1002/jbt.20064

18. Maiti S, Zhang J, Chen G. Redox regulation of human estrogen sulfotransferase (hSULT1E1). *Biochem Pharmacol*. 2007;73(9):1474-1481. doi:10.1016/j.bcp.2006.12.026

19. Bouchentouf, Salim; Missoum, Noureddine (2020): Identification of Compounds from Nigella Sativa as New Potential Inhibitors of 2019 Novel Coronasvirus (Covid-19): Molecular Docking Study.. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.12055716.v1

20. Angeletti S, Benvenuto D, Bianchi M, Giovanetti M, Pascarella S, Ciccozzi M. COVID-2019: The role of the nsp2 and nsp3 in its pathogenesis [published online ahead of print, 2020 Feb 21]. *J Med Virol*. 2020;10.1002/jmv.25719. doi:10.1002/jmv.25719

21. Luo H, Ye F, Sun T, et al. In vitro biochemical and thermodynamic characterization of nucleocapsid protein of SARS. *Biophys Chem*. 2004;112(1):15-25. doi:10.1016/j.bpc.2004.06.008

22. Tabassum H, Ahmad IZ. Molecular Docking and Dynamics Simulation Analysis of Thymoquinone and Thymol Compounds from Nigella sativa L. that Inhibits Cag A and Vac A Oncoprotein of Helicobacter pylori: Probable Treatment of H. pylori Infections [published online ahead of print, 2020 Mar 1]. *Med Chem*. 2020;10.2174/1573406416666200302113729. doi:10.2174/1573406416666200302113729

23. Banerjee Amrita, Santra D and Maiti S. Energetics based epitope screening in SARS CoV-2 (COVID 19) spike glycoprotein by Immuno-informatic analysis aiming to a suitable vaccine development. bioRxiv 2020.04.02.021725; doi: https://doi.org/10.1101/2020.04.02.021725

24. Maiti, S.; Banerjee, A. Epigallocatechin-Gallate and Theaflavin-Gallate Interaction in SARS CoV-2 Spike-Protein Central-Channel with Reference to the Hydroxychloroquine Interaction: Bioinformatics and Molecular Docking Study. Preprints 2020, 2020040247 (doi: 10.20944/preprints202004.0247.v1).

25. Mokhtari-Zaer A, Norouzi F, Askari VR, et al. The protective effect of Nigella sativa extract on lung inflammation and oxidative stress induced by lipopolysaccharide in rats. *J Ethnopharmacol*. 2020;253:112653. doi:10.1016/j.jep.2020.112653

26. Mohebbati R, Abbasnezhad A. Effects of Nigella sativa on endothelial dysfunction in diabetes mellitus: A review. *J Ethnopharmacol*. 2020;252:112585. doi:10.1016/j.jep.2020.112585

27. Babar ZM, Jaswir I, Tareq AM, et al. *In vivo* anxiolytic and *in vitro* anti-inflammatory activities of water-soluble extract (WSE) of *Nigella sativa* (L.) seeds [published online ahead of print, 2019 Oct 3]. *Nat Prod Res*. 2019;1–6. doi:10.1080/14786419.2019.1667348

28. Eladl AH, Arafat N, El-Shafei RA, Farag VM, Saleh RM, Awadin WF. Comparative immune response and pathogenicity of the H9N2 avian influenza virus after administration of Immulant[®], based on Echinacea and Nigella sativa, in stressed chickens. *Comp Immunol Microbiol Infect Dis*. 2019;65:165–175. doi:10.1016/j.cimid.2019.05.017

29. Mahboubi M. Natural therapeutic approach of Nigella sativa (Black seed) fixed oil in management of Sinusitis. Integrative Medicine Research, 02 Feb 2018, 7(1):27-32 DOI: 1016/j.imr.2018.01.005PMID: 29629288 PMCID: PMC5884000

30. Razin MAF, Osman A, Ali MA, Bahgat MM, Maghraby AS. Immune responses to killed reassorted influenza virus supplemented with natural adjuvants. *Acta Microbiol Immunol Hung*. 2017;64(3):313–330. doi:10.1556/030.64.2017.011

31. Onifade AA, Jewell AP, Adedeji WA. Nigella sativa concoction induced sustained seroreversion in HIV patient. *Afr J Tradit Complement Altern Med*. 2013;10(5):332–335. Published 2013 Aug 12.

Conflict Of Interest

None

Table 1

Table 1. Autodock results on the binding energies of nigellidine and several important SARS CoV-2 proteins and some human inflammatory receptors i.e. IL1 receptor IL1R and TNFα receptors TNFR1 and TNFR2

Figures

Energy values are kcal/mol.

nsp2_QHD4345_2

Binding Energy	Ligand Efficiency
-6.28	-0.29
-5.86	-0.27
-4.46	-0.2
-4	-0.18

nucleocapsid_QHD43423

Binding Energy	Ligand Efficiency
-6.24	-0.28
-6.05	-0.27
-4.9	-0.22

Mpro/3CLpro_QHD43415_5

Binding Energy	Ligand Efficiency
-6.38	-0.29
-6.18	-0.28
-4.19	-0.19

N-terminus_protenase QHD43415_3

Binding Energy	Ligand Efficiency
-7.61	-0.35
-7.59	-0.35
-6.18	-0.28

IL1R_1itb

Binding Energy	Ligand Efficiency
-6.23	-0.28
-5.86	-0.27
-5.67	-0.26
-5.63	-0.26
-5.6	-0.25
-5.56	-0.25
-5.34	-0.24
-5.23	-0.24
-4.8	-0.22
-4.77	-0.22

TNFR1_1ncf

Binding Energy	Ligand Efficiency
-6.81	-0.31
-6.81	-0.31
-6.74	-0.31
-6.72	-0.31
-6.7	-0.3
-6.57	-0.3
-6.56	-0.3
-6.55	-0.3

Legend:
- Active site (yellow)
- Secondary active site (green)
- Other site (white)

TNFR2_3alq_D

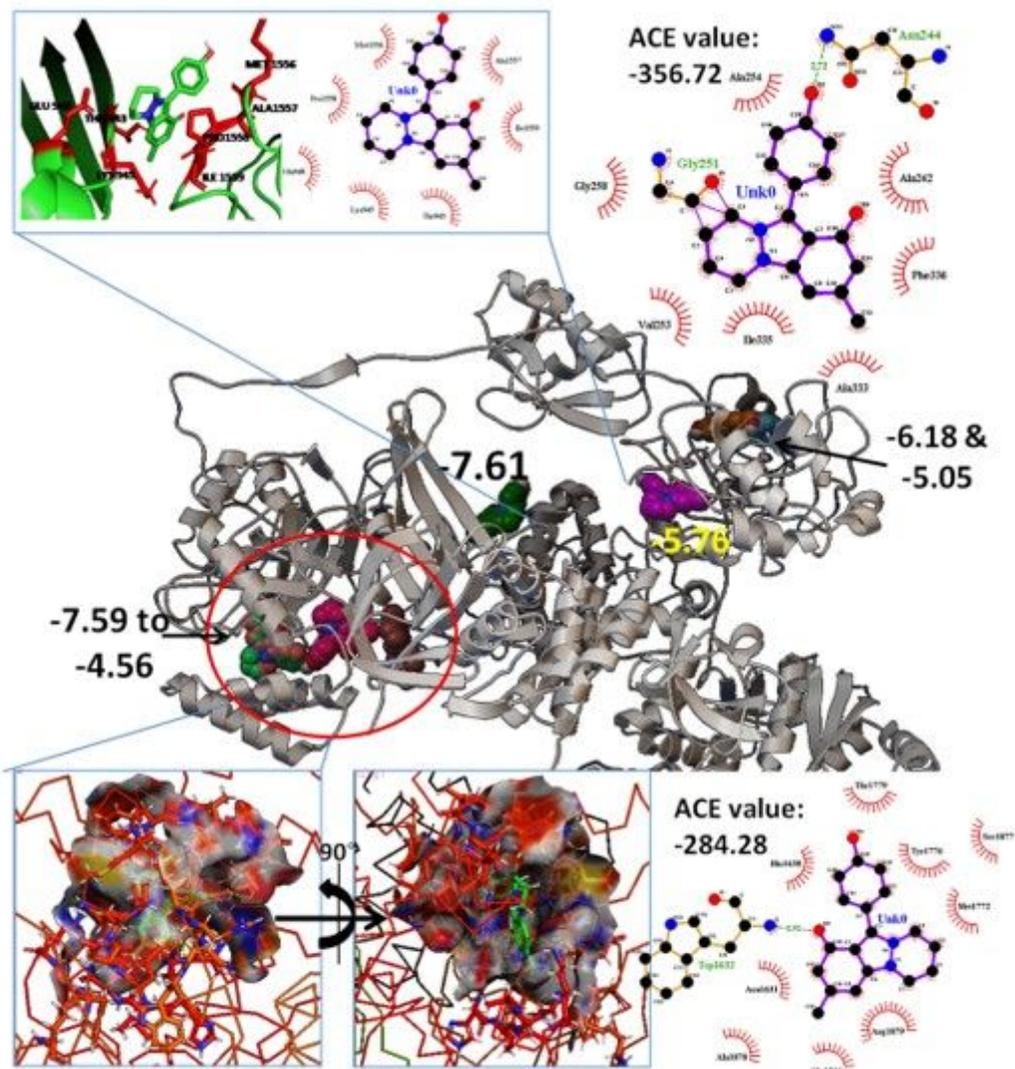| Binding Energy	Ligand Efficiency
-5.16	-0.23
-5.13	-0.23
-5	-0.23

Figure 1

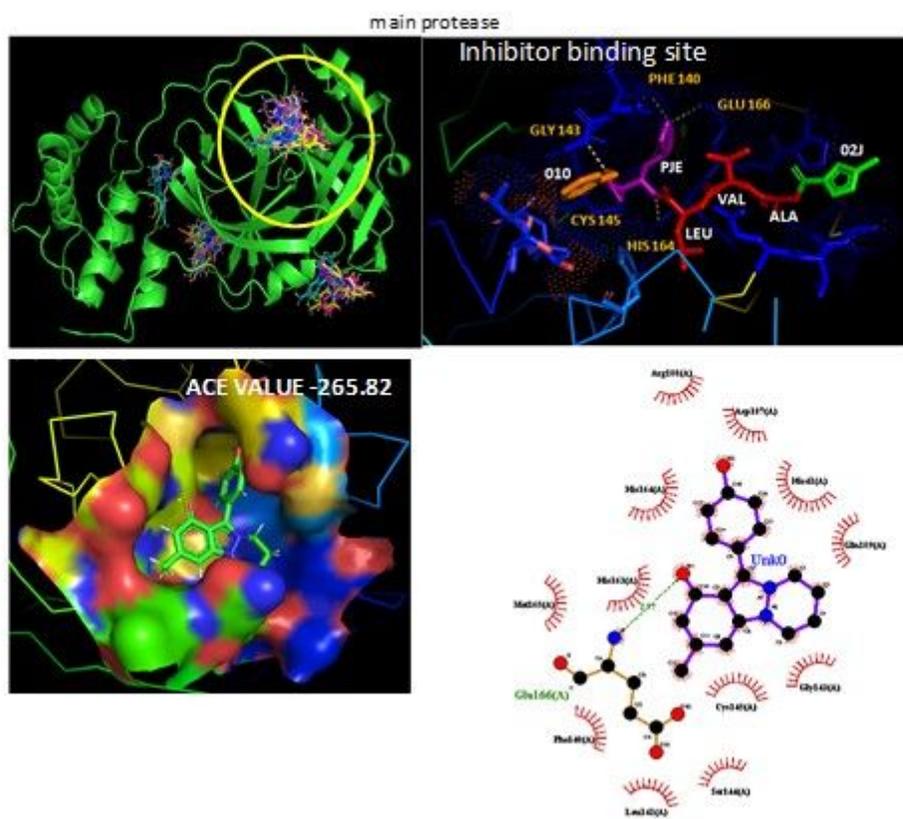Interaction of nigellidine with N-terminal protease of SARS CoV-2

Figure 2

Interaction of nigellidine with 6LU7_The crystal structure of COVID-19 main protease (ACE = -265.82)
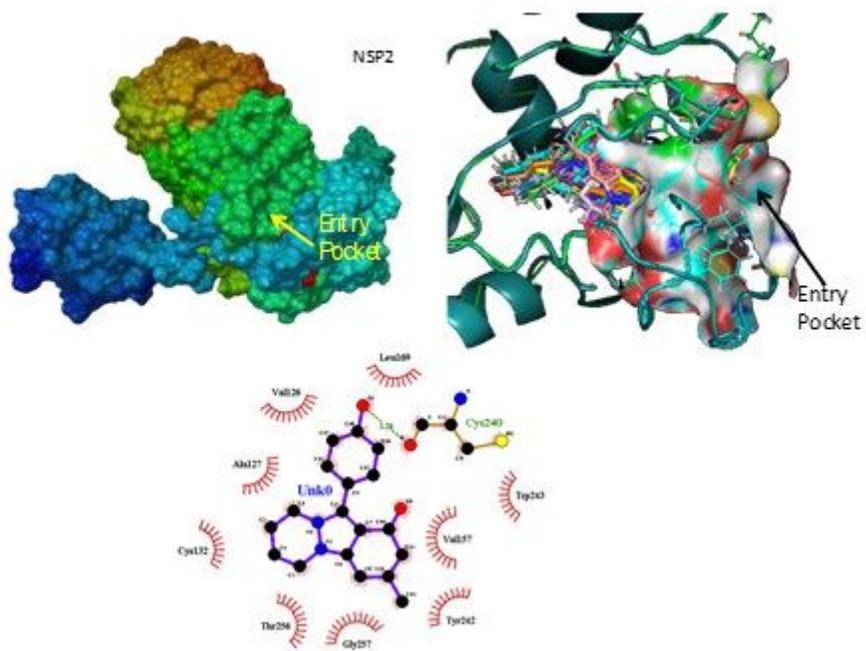
Figure 3

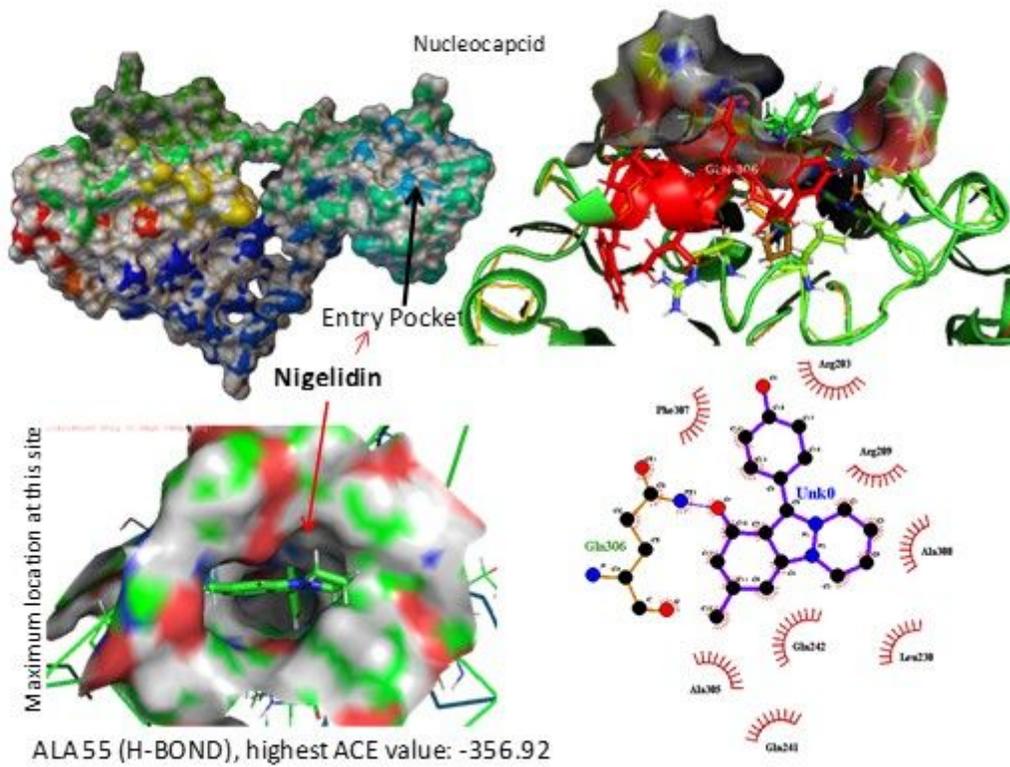Interaction of nigellidine with NSP2, CYS 240 (ACE value: -244.81)

Figure 4

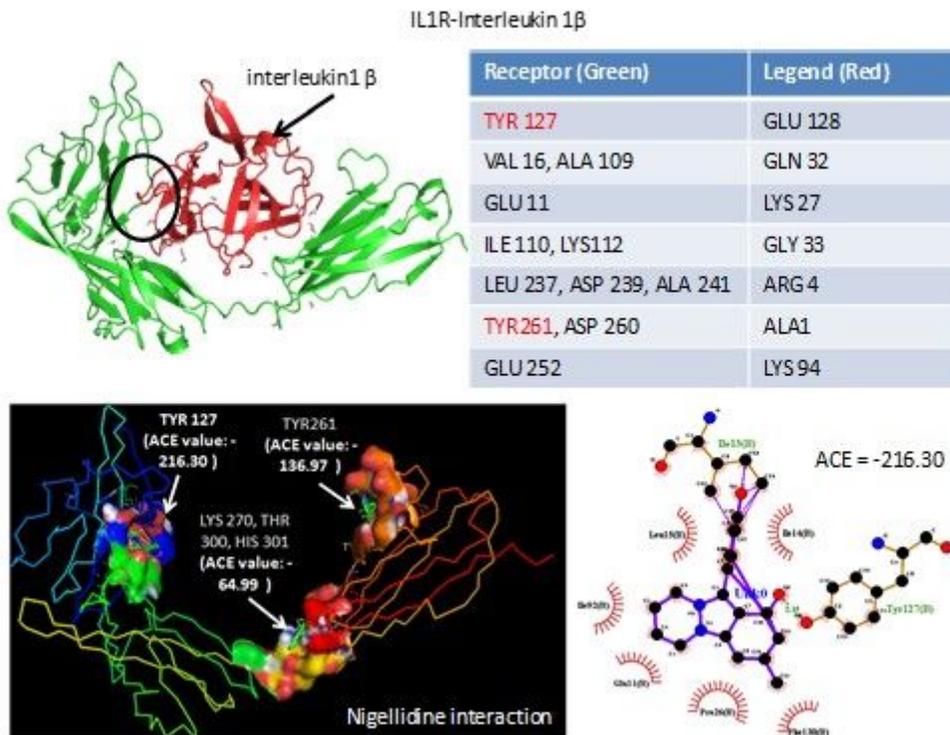Interaction of nigellidine with Nucleocapcid

IL1R-Interleukin 1β

Receptor (Green)	Legend (Red)
TYR 127	GLU 128
VAL 16, ALA 109	GLN 32
GLU 11	LYS 27
ILE 110, LYS112	GLY 33
LEU 237, ASP 239, ALA 241	ARG 4
TYR261, ASP 260	ALA1
GLU 252	LYS 94

Nigellidine interaction

ACE = -216.30

Figure 5

Interaction of nigellidine with IL1R. Common TYR127 and TYR261 are involved to both interactions IL1 and the drug nigellidine.
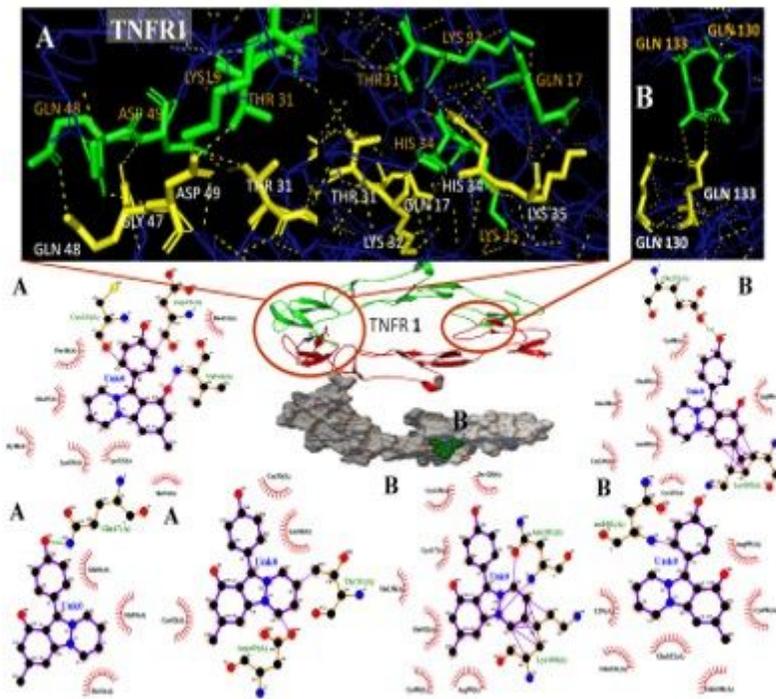
Figure 6

Interaction of nigellidine with TNFR1. Site A and B representing the amino acid interactions within TNFR1 and also nigellidine binding at the same site.
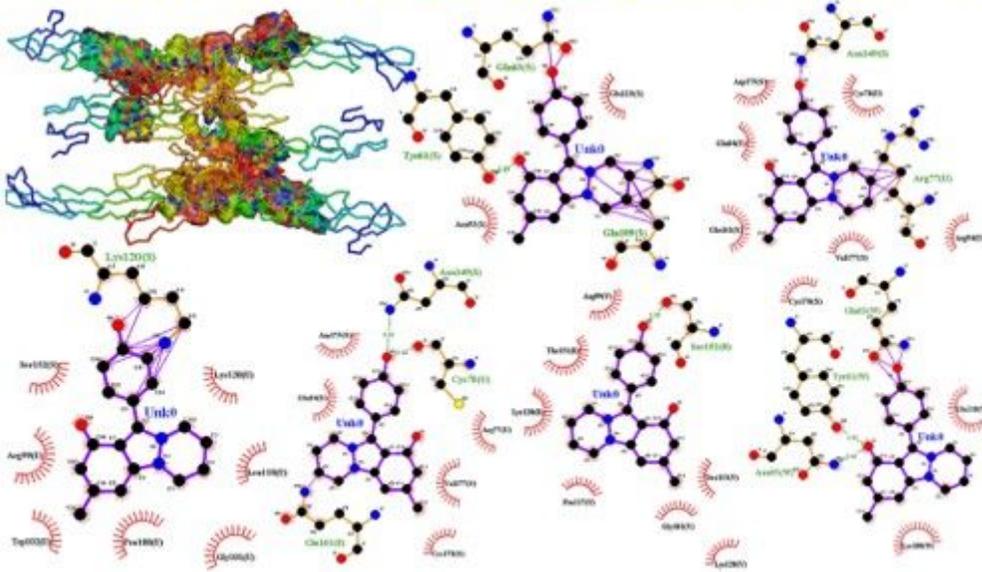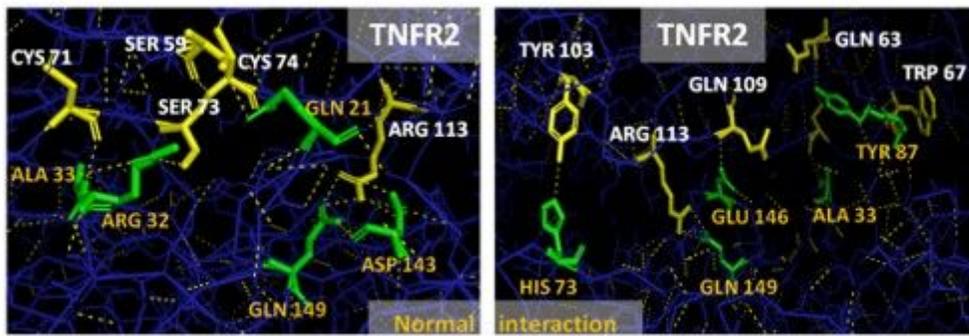
Figure 7

Interaction of nigellidine with TNFR2. Nigellidine formed bonds with GLN63 and GLN109.
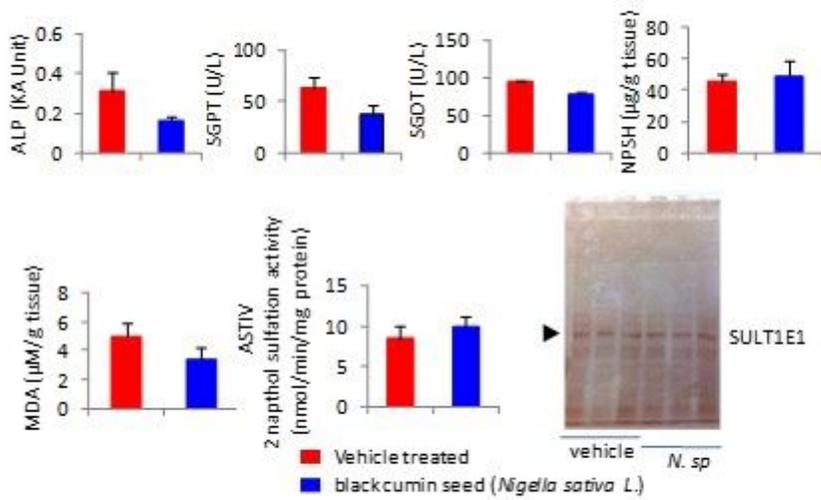
Figure 8

Effects of NS treatment on hepatic functions and antioxidant status in experimental rat model. Drug metabolizing enzymes ASTIV and EST/SULT1E1 activity/expression has been checked in the rat liver in response to NS extract exposure.