International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2020; 8(5): 01-09
Received: 25-06-2020
Accepted: 10-07-2020

Manjunath NS
Department of Biotechnology, Bapuji Institute of Engineering and Technology, Davangere, Karnataka, India

Rangaswamy BE
Department of Biotechnology, Bapuji Institute of Engineering and Technology, Davangere, Karnataka, India.

Hafsa Javeed
Department of Biotechnology, Bapuji Institute of Engineering and Technology, Davangere, Karnataka, India

Ganavi D
Department of Biotechnology, Bapuji Institute of Engineering and Technology, Davangere, Karnataka, India

Sahana JK
Department of Biotechnology, Bapuji Institute of Engineering and Technology, Davangere, Karnataka, India

UllasKotihal
Department of Biotechnology, Bapuji Institute of Engineering and Technology, Davangere, Karnataka, India

Corresponding Author:
Manjunath NS
Department of Biotechnology, Bapuji Institute of Engineering and Technology, Davangere, Karnataka, India

Evaluation of *Nigella sativa* (Black cumin) for anti-cancer and anti-inflammatory activities

Manjunath NS, Rangaswamy BE, Hafsa Javeed, Ganavi D, Sahana JK and Ullas Kotihal

Abstract
Natural products obtained from plants are used to treat various diseases in folk medicine including Cancer and inflammation. *Nigella sativa*is annual flowering plants that appertain to Ranunculaceae family. Numerous studies have shown that naturally occurring compounds may support prevention and treatment of various diseases. The aim of our study is to evaluate the cytotoxicity studies for AGS and PANC^{-1} cell lines and Anti-inflammatory study using RAW264.7 cell line. The *N.sativa* oil is extracted by soxhelt extraction by using petroleum ether as the whole solvent. For cytotoxicity study the MTT assay is done, which is simple, accurate and yield reproducible result, cytotoxicity is the quality of being toxic to cell. The activity of RAW264.7 cells against iNOS release post LPS stimulation, which is observed calorimetrically. Hence by the graphical studies we can conclude that the crude of *N. sativa* is consequential for our successive evaluation of Anticancer and Anti-inflammatory modalities.

Keywords: *Nigella sativa*, black cumin, anticancer, ranunculaceae, anti-inflammatory, soxhlet extraction, petroleum ether

1. Introduction
Plants have long been used as a basis of traditional remedies in the history of mankind and also act as sources of modern medicines. According to the World Health Organization (WHO), more than three-fourths of the communities in resource-limited countries rely upon medicinal plants for their primary health care needs because more than 60% of the society isenable to have access and/or afford allopathic medicines. The development of new products from natural sources is also encouraged because it is estimated that, of the 300,000 herbal species that exist globally, only 15% have been explored for their pharmacological potential [1]. Among several medicinal plants, *Nigella sativa* L. (Ranunculaceae) has been considered one of the most treasured nutrient-rich herbsin history around the world and numerous scientific studies are in progress to validate the traditionally claimed uses of small seed of this species. The maximal nutritional value of black cumin can be linked to the presence of substantial amount of vegetable protein, fiber and minerals, and vitamins. The nutritional composition reported from different sources revealed 20-85% of protein, 38.20% of fat, 7-94% of fiber, and 31.94% of total carbohydrates [1]. Among various amino acids identified glutamate, arginine, and aspartate while cysteine and methionine were the major and minor amino acids, respectively. Black cumin seeds also contain significant levels of iron, copper, zinc, phosphorus, calcium, thiamin, niacin, pyridoxine, and folic acid. Black cumin essential oil is a valuable source of bioactive compounds including *p*-cymene, thymoquinone, *α*-thujene, longifolene, *β*-pinene, *α*-pinene and carvacrol [2]. In addition, phytochemical analyses of *N. sativa* displayed the presence of over hundreds of phytoconstituents which include mainly alkaloids, saponins, sterols, and essential oil but the composition of many of these have not been chemically recognized nor have been biologically verified. The *N. sativa* seed contain 26-34% fixed oil of which the major fatty acids are linoleic acid (64.6%) and palmitic acid (20.4%). The seed oil is comprised of 0.4%–2.5% essential oil [1]. Black cumin seed have two different forms of alkaloids: isoquinoline alkaloid that includes: nigellicimine, nigellicimine n-oxide and pyrazol alkaloid that includes: nigellidine and nigellicine [3]. Amongst different active constituents reported so far, thymoquinone found as major component of the essential oil and is the bioactive compound, exhibits wide ranging therapeutic benefits. The seed of *Nigella sativa* has been used in different civilization around the world for centuries to treat various animal and human ailments. So far, numerous studies demonstrated the seed of *Nigella sativa* and its main active constituent, thymoquinone, to be medicinally very effective against various illnesses including different chronic illness: neurological and mental illness, cardiovascular disorders, cancer, diabetes, inflammatory conditions and infertility as well as various infectious diseases

due to bacterial, fungal, parasitic, and viral infections. Besides, when combined with different conventional chemotherapeutic agents, it synergizes their effects resulting in reducing the dosage of concomitantly used drugs with optimized efficacy and least and/or no toxicity [1]. In a number of old cultures, *N. sativa* was used as a spice, preservative, food additive, and herbal remedy for numerous diseases, such as asthma, diarrhea, diabetes, headache, toothache, nasal congestion, and several types of cancers [4].

Thymoquinone (TQ), the main pharmacological active ingredient within the black cumin seed is believed to be responsible for the therapeutic effects on chronic inflammatory role of TQ in lipopolysaccharide (LPS) – stimulated BV-2 murine microglial cells. The results obtained in studies indicate that TQ was effective in reducing NO_2 (-) with an IC50 of 5.04µM, relative to selective iNOS inhibitor LNIL-I-N6-(1-iminoethyl) lysine (IC50 4.09µM). TQ mediated reduction in NO_2 (-) was found to parallel the decline of iNOS protein expression as confirmed by immune cytochemistry. In addition, TQ was found to reduce LPS mediated elevation in gene expression of Cxcl10 and a number of other cytokines in the panel. These findings demonstrate the significant anti-inflammatory properties of TQ in LPS activated microglial cells. Therefore, the obtained results might indicate the usefulness of TQ in delaying the onset of inflammation-mediated neurodegenerative disorders involving activated microglia cells [4].

A number of pharmaceutical and biological properties have been ascribed to seeds of *N. sativa*. The present review focuses on the profile of high-value components along with traditional medicinal and biological principles of *N. sativa* seed and its oil so as to explore functional food and nutraceutical potential of this valued herb.The effectiveness of *N. sativa* against cancer in the blood system, kidneys, lungs, prostate, liver, and breast and on many malignant cell lines has been shown in many studies, but the molecular mechanisms behind that anti-cancer role are still not clearly understood. From among the many effects of *N. sativa*, including its anti-proliferative effect, cell cycle arrest, apoptosis induction, ROS generation, anti-metastasis/anti-angiogenesis effects, Akt pathway control, modulation of multiple molecular targets, including p53, p73, STAT-3, PTEN, and PPAR-*γ*, and activation of caspases, the main suggestive anti-cancer mechanisms of *N. sativa* are its free radical scavenger activity and the preservation of various anti-oxidant enzyme activities, such as glutathione peroxidase, catalase, and glutathione-S-transferase [5].

2. Materials and methods

2.1 Chemicals and Consumables

Methanol, Petroleum ether, 3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) in powdered form (the solution is filtered through a 0.2µm filter and stored at 2–8 °C for frequent use or frozen for extended periods), DMSO (Dimethlysulfoxide), DMEM (Dulbecco's Modified Eagle Medium), FBS (Fetal Bovine Serum), Penicillin, Streptomycin, EDTA and Tripsin. Stock cultures of AGS, PANC-1 and RAW 264.7 cell lines were procured from ATCC (American Type Culture Collection).

2.2 Extraction Process

2.2.1 Solvent Extraction

Preparation of Extract:

1. Weighed 20g of dried sample powder and dissolved in 100ml of methanol in 500ml beaker with aluminum foil covered on it.
2. Then the beaker was kept on hot water bath at 50º C for 4 hours.
3. After incubation period the extract was filtered with filter paper (Whatman filter paper) and the filtrate was collected in 50ml beaker. Residue present over the filter paper was discarded and filtrate was taken for further use [6].
4. Filtrate was kept at 50ºC for few hours until the extract got completely dried and turned into semisolid form.
5. This semi solid sample was weighed and the yield was noted.

Table 1: Yield summary after crude extraction

Sample	Sample Taken for extraction	Solubility	Yield
Black Cumin extract	20g	MeOH	1022mg

2.2.2 Soxhlet Extraction

1. The seeds were purchased, nicely cleaned and air dried.
2. The dried samples were powdered with the help of grinder or mixer. The powdered sample was kept in an air tight container away from light until use.
3. Extraction of oil is done by the method hot solvent extraction by soxhlet technique. Soxhlet extraction is the process of transferring the partially soluble components of a solid to the liquid phase using a Soxhlet extractor.
4. The solid is placed in a filter paper thimble which is then placed into the main chamber of the Soxhlet extractor.
5. The solvent (heated to reflux) travels into the main chamber and the partially soluble components are slowly transferred to the solvent.
6. Hot solvent extraction was done using a reflux apparatus for 48 h at 40 °C [7].
7. The solvent used above is Petroleum ether (Fisher Scientific, Petroleum spirit 40°C - 60°C).

Table 2: Effect of different solvents on yield and Thymoquinone content

Extraction Method	Solvent	Extraction Yield (%w/w)	Thymoquinone (%w/w)
Hot solvent extraction by Soxhlet Technique	Petroleum ether	32.7±5.1	3.66±1.43
	Methanol	40.1±8.5	6.77±1.2

Fig 1: Soxhlet apparatus

Fig 2: Soxhlet chamber

2.3 Anticancer activity

2.3.1 Cytotoxicity studies for AGS and PANC-1 cell line

Cancer is a rapidly growing health problem and posing a serious challenge to health professionals and researchers. American cancer society has reported that more than 0.5 million deaths occur due to cancer during 2013 in USA alone and the numbers are really threatening in under-developed world. With the wide spread and devastating effect of cancer and its high economic load, there is need for identifying natural and cheap products with anti-carcinogenic activity. Many studies depicts that risk of cancer occurrence can be reduced by the consumption of many vegetables and fruits. *N. sativa* is one of them who showed promising anti-cancer activity in number of studies [8]. Gastric cancer is a very aggressive tumor and represents the second leading cause of cancer mortality worldwide. Systemic chemotherapy alone remains to be the mainstay strategy in cancer treatment, but resistance to chemotherapeutic agents appears and patients die in a short period of time [9].

Traditionally, the *in vitro* determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity [10]. The major terpenes, thymoquinone (TQ), dithymoquinone (DTQ), trans-anethol, p-cymene, limonine, and carvone have been identified in *Nigella sativa*. The TQ and DTQ found in black cumin are both cytotoxic for various types of tumors [11]. For many years, an extract of *N. sativa* seeds, *Smilax glabra* (rhizome) and *Hemidesmus indicus* (root), has been used for the treatment of cancer in Sri Lanka [5].

The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red.

Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured.

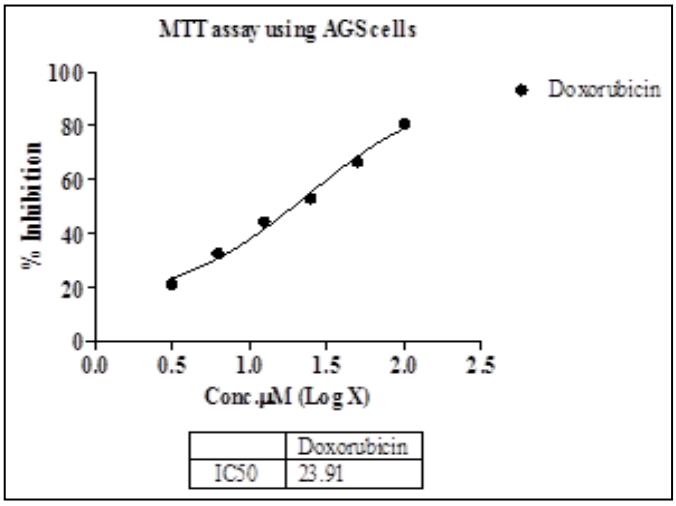


Fig 3: Graphical representation of various concentration of Doxorubicin Standard vs % Inhibition in AGS cells

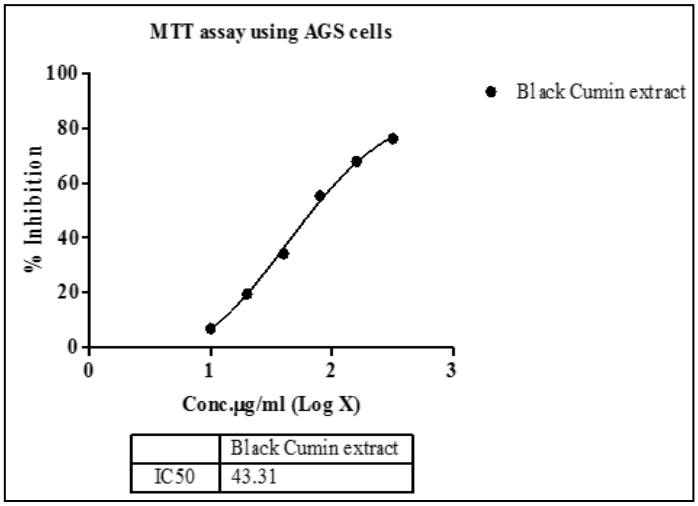


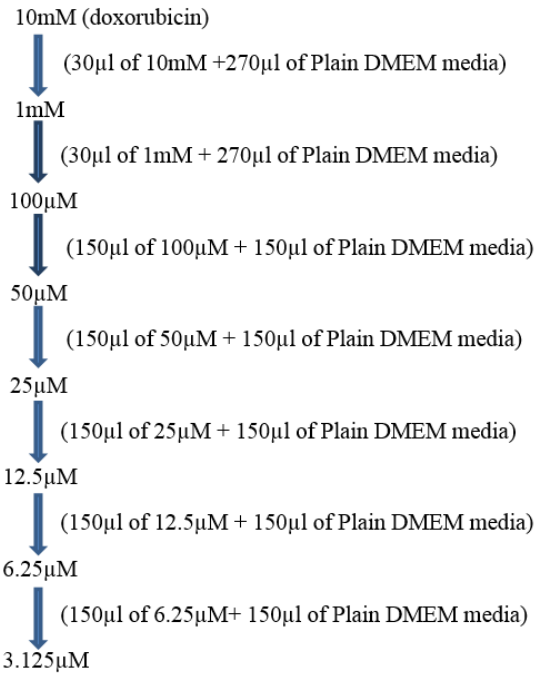
Fig 4: Graphical representation of various concentration of Black cumin extract vs % Inhibition in AGS cells

2.4 Materials & methods

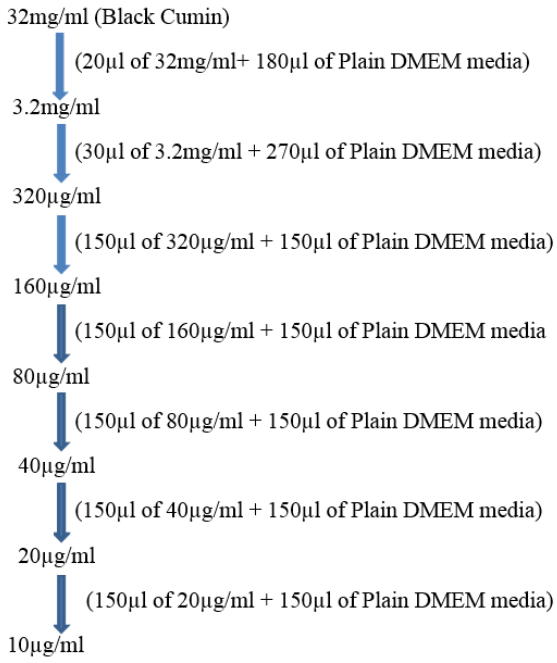
1. MTT Powder (the solution is filtered through a 0.2µm filter and stored at 2–8 °C for frequent use or frozen for extended periods)
2. DMSO
3. CO_2 incubator
4. Spectramax i3x plate reader

2.4.1 Preparation of test solutions

2.4.2 Standard: Doxorubicin - 10mM stock of Doxorubicin was taken. Further Serial two fold dilutions were prepared from 100µM to 3.125µM using DMEM plain media for treatment.



2.4.3 Sample preparation: For cytotoxicity studies, 32mg/ml stocks were prepared using DMSO. Serial two fold dilutions were prepared from 320µg/ml to 10µg/ml using DMEM plain media for treatment.



2.4.4 Cell lines and culture medium:
AGS and PANC-1 cell lines was procured from ATCC, stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent. The cell was dissociated with cell dissociating solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The viability of the cells is hecked and centrifuged. Further, 50,000 cells /well was seeded in a 96 well plate and incubated for 24 hrs. at 37ºC, 5% CO_2 incubator.

2.4.5 Source of reagents: DMEM, FBS, PenStrep, Trypsin-procured from Invitrogen.

2.4.6 Procedure
The monolayer cell culture was trypsinized and the cell count was adjusted to $5x10^5$ cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37ºC for 24hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37º C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line.

Calculating Inhibition
% Inhibition= ((OD of Control – OD of Sample) / OD of Control) x 100

Statistical evaluation:
IC50 Value
The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half.

Nonlinear regression
In statistics, nonlinear regression is a form of regression analysis in which observational data are modelled by a function which is a nonlinear combination of the model parameters and depends on one or more independent variables. The data are fitted by a method of successive approximation.

2.5. Anti-inflammatory activity
2.5.1 Anti-inflammatory studies using raw 264.7 cells
The inflammation is a sequence of events that occurs in response to noxious stimuli, infection, trauma, or injury in the living tissues.Inflammation is an important component of the physiological response to harmful stimuli such as infection. Acute and prolonged inflammatory events perform essential roles in promoting innate immunity, maintaining immune system homeostasis, and as a natural defense mechanism [12]. The inflammation is initiated by a cascade of events including enzyme activation, media to srelease, fluid extravasations, cell migration, tissue breakdown and repair processes. The inflammation releases white blood cells as a protective measure against injury. These white blood cells synthesize several biomolecules and release them after injury leading to swelling and redness [13].For centuries, particularly in the Middle East, black cumin seeds (*Nigella sativa*) have been used to treat a diverse range of peripheral and central inflammatory related illnesses such as diabetes, asthma, arthritis, colitis, periodontitis and encephalomyelitis.

Moreover, unlike some synthetic drugs, the anti-inflammatory properties of black cumin seeds are accompanied by a milieu of health promoting effects on almost every major system (i.e. cardiovascular, respiratory, immune) and organ (i.e. digestive tract, kidney, pancreas and liver) of the human body. The ability of TQ to block NF-kappaB activation and its molecular targets can account for much of its anti-neuro inflammatory effects which are strengthened by ability to attenuate cytokines including TNF-alpha, IL-1beta, nitric oxide (NO) / iNOS, IL-6, IFN-gamma, prostaglandin E2, TGF-beta1, 5-lipoxygenase activity and cyclooxygenase-2. Heightened biological activity of these processes are associated with age related neurodegenerative diseases often perpetuated by chronic CNS inflammation via resident macrophages in the brain; glial/ microglia cells [4].

Principle: Nitric oxide (NO) is a short-lived free radical that mediates many biological processes. One of the functions of NO is to enhance the bactericidal and tumoricidal activities of activated macrophages. Excessive production of NO could however potentially lead to tissue damage and activation of pro-inflammatory mediators. The potential of extracts from medicinal plants to scavenge these free radicals and modulate inflammatory reactions has been demonstrated.

2.5.2 Procedure

The murine monocytic macrophage RAW 264.7 cell line was cultured in Dulbecco's Modified Eagle Media (DMEM) (2mM L-glutamine, 45g/L glucose, 1mM sodium pyruvate) with 10% fetal bovine serum (FBS). The cells were cultured at 37°C with 5% CO_2 and were sub-cultured twice a week. The cells were seeded in 96-well tissue culture plates ($1x10^6$cells/ml) and incubated for 24 h at 37°C with 5% CO_2. Then, 100µl of test extract in DMSO was serially diluted to give two-fold concentration variations and then added onto cells. Cells were then stimulated with and 10µg/ml *E.coli* lipopolysaccharide (LPS) and incubated at 37°C for another 17h. After 24 h incubation, 100µL of supernatant from each well of cell culture plates was transferred into96-well micro-titre plates and equal volume of Griess reagent was added. The absorbance of the resultant solutions in the wells of the microtiter plate was determined with a micro-titre plate reader (Spectramax i3x) after 10min at 550nm. The concentrations of nitrite were calculated from regression analysis using serial dilutions of sodium nitrite as a standard. Percentage inhibition was calculated based on the ability of extracts to inhibit nitric oxide formation by cells compared with the control (cells in media without extracts containing triggering agents and DMSO), which was considered as 0% inhibition.

3. Results & discussion

3.1 Anticancer

3.1.1 Cytotoxicity studies for AGS cell lines

Here the control, doxorubicin and black cumin extract's OD (optical density) is measured, rate of inhibition is calculated. After which a graph is plotted between% of inhibition (rate of inhibition) and concentration to calculate IC50 value of both doxorubicin and black cumin extract. The cytotoxicity level is measured on both AGS and PANC-1 cell lines.

Table 3: Colorimetric reading of standard doxorubicin of various

AGS				
Sample	**Conc. (µM)**	**OD @ 590nm**	**% Inhibition**	**IC50 (µM)**
Control	0	0.523	0.00	23.91
Doxorubicin	3.125	0.412	21.22	
	6.25	0.352	32.70	
	12.5	0.291	44.36	
	25	0.245	53.15	
	50	0.174	66.73	
	100	0.101	80.69	

Concentrations and its% inhibition on AGS cell line

Table 4: Colorimetric reading of Black cumin extracts of various

AGS				
Sample	**Conc (µg/ml)**	**OD @ 590nm**	**% Inhibition**	**IC50 (µg/ml)**
Control	0	0.523	0.00	43.31
Black Cumin extract	10	0.487	6.88	
	20	0.421	19.50	
	40	0.344	34.23	
	80	0.234	55.26	
	160	0.168	67.88	
	320	0.125	76.10	

Concentrations and its% inhibition on AGS cell lines

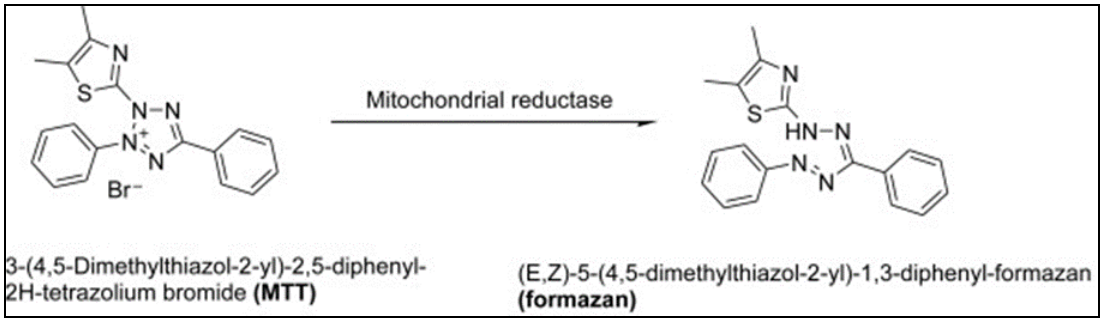
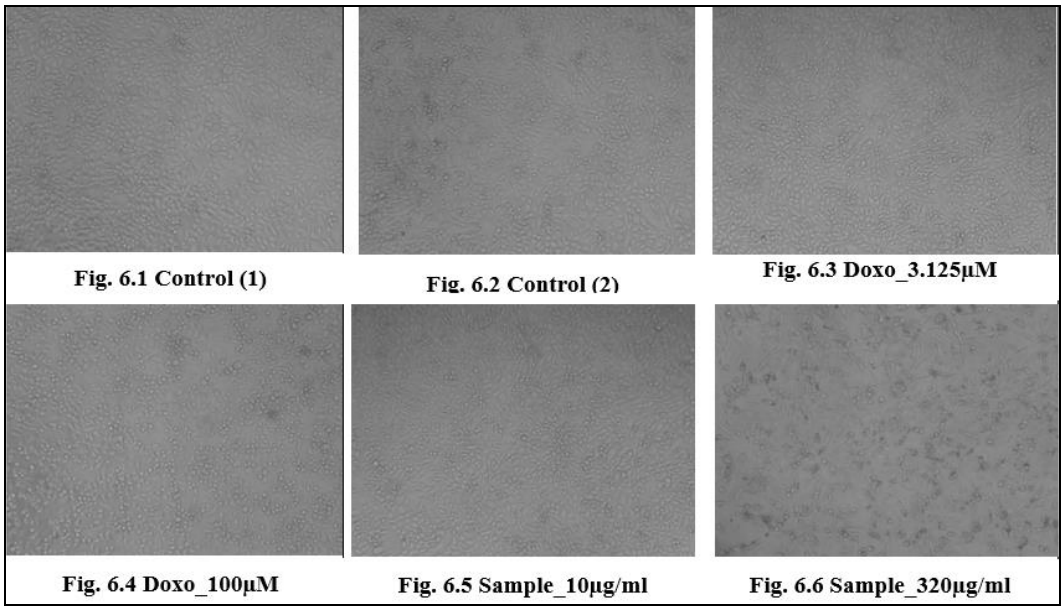


Fig 5: Enzymatic reduction of MTT to formazan.



(AGS cell lines)

Fig 6: Effect of Doxorubicin (std) Black cumin extract (sample) on AGS cells

Various concentration of Black cumin extractwas treated with AGS cell lines. After 24hrs of incubation, a significant difference was observed between control and highest concentration of black cumin extract used 320µg/ml. It showed maximum percentage growth inhibition can be seen in the Fig 8.2. Hence the black cumin is effective in decreasing the cytotoxicity of gastric cancer cells.

3.1.2 Cytotoxicity studies for PANC-1 cell lines

Table 5: Colorimetric reading of Standard Doxorubicin of various

PANC-1				
Sample	**Conc (µM)**	**OD @ 590nm**	**% Inhibition**	**IC50 (µM)**
Control	0	0.684	0.00	19.54
Doxorubicin	3.125	0.489	28.51	
	6.25	0.434	36.55	
	12.5	0.381	44.30	
	25	0.296	56.73	
	50	0.214	68.71	
	100	0.175	74.42	

Concentration and its% inhibition on PANC-1 cell line

Table 6: Colorimetric reading of Black cumin extract of various

PANC-1				
Sample	**Conc. (µg/ml)**	**OD @ 590nm**	**% Inhibition**	**IC50 (µg/ml)**
Control	0	0.684	0.00	144.4
Black Cumin extract	10	0.621	9.21	
	20	0.563	17.69	
	40	0.530	22.51	
	80	0.474	30.70	
	160	0.301	55.99	
	320	0.257	62.43	

Concentration and its% inhibition on PANC-1 cell lines

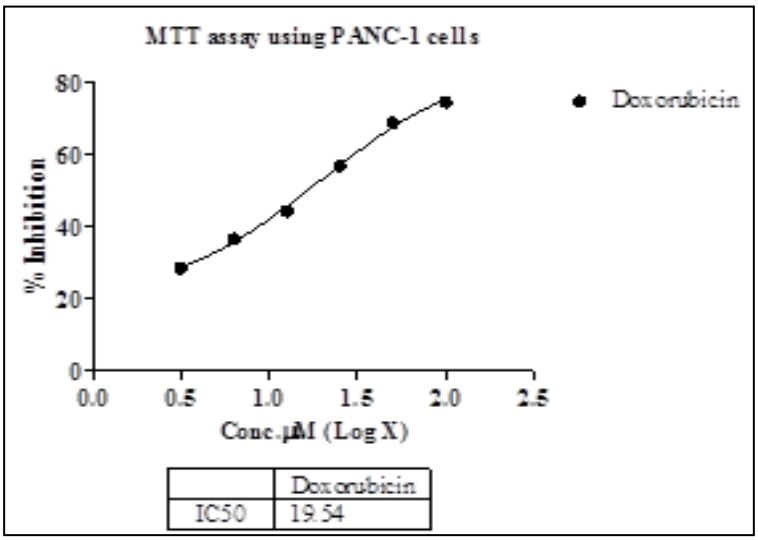


Fig 7: Graphical representation of various concentration of Doxorubicin Standard vs% Inhibition

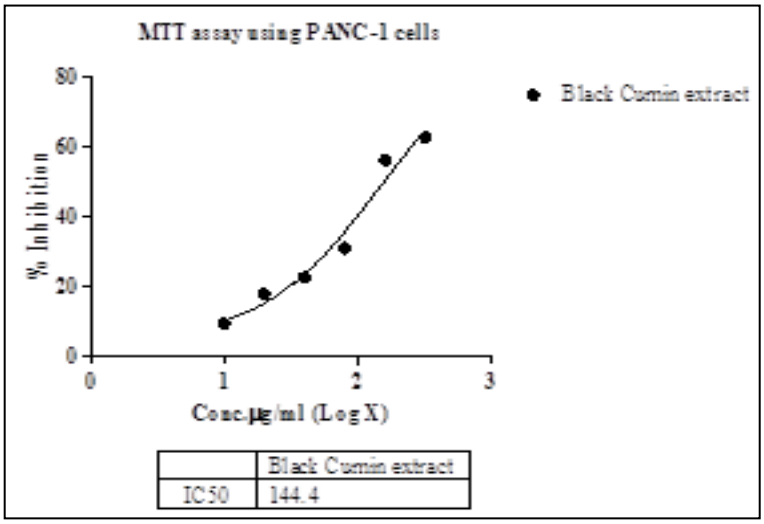
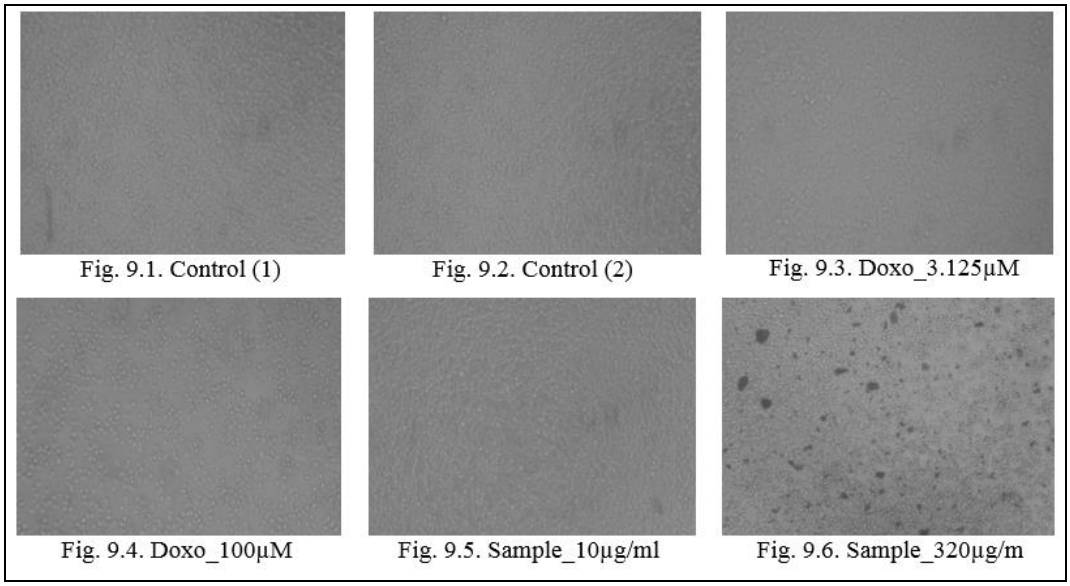


Fig 8: Graphical representation of various concentration of Black cumin extract vs% Inhibition



(PANC-1 cell lines)

Fig 9: Effect of Doxorubicin (Std) and Black cumin extract (sample) on PANC-1 Cell lines

Morphology of PANC-1 cells when exposed to various concentration of Black cumin extract (0-320µg/ml) was shown in fig 11.4. A significant difference was observed between control and highest concentration of black cumin extract. Thus, black cumin also helps in decreasing the growth of pancreatic cancer cells. Many of the anti-cancer activities of *N. sativa* have been attributed to its major active constituent, thymoquinone (TQ). TQ has been shown to exert

anti-proliferative, pro-apoptotic, anti-oxidant, anti-oxidant, anti-mutagenic, anti-angiogenic, and anti-metastatic effects against cancer cells. TQ seems to mediate its anti-cancer effects by targeting a number of cellular pathways involving p53, NF-κB, PPARγ, STAT3, MAPK, and PI3K/AKT transducing signals. Besides TQ, other phytoconstituents of *N. sativa* have also been shown to contribute to the anti-cancer potential of *N. sativa* extracts. α-hederin is a pentacyclic triterpene saponin found in *N. sativa* seeds that exerts effective anti-cancer effects, both *in vitro* and *in vivo*. Moreover, thymol, thymohydroquinone, dithymoquinone, nigellimine-N-oxide, nigellicine, nigellidine, and carvacrol are phytoconstituents of *N. sativa* that have been demonstrated to play anti-cancer and cytotoxic functions. Yet, the exact molecular mechanisms underlying the anti-cancer effects of these phytoconstituents are not fully known, and future studies are needed to elucidate the detailed mechanisms of action that mediate the anti-cancer effects of *N. sativa* phytoconstituents [14].

3.2 Anti-inflammatory

Anti-inflammatory studies using raw 264.7 cells

Here the control and black cumin extract's OD (optical density) is measured and rate of inhibition is calculated. The cell lines used to evaluate anti-inflammatory activity are Raw 264.7.

Table 7: Colorimetric reading of Black cumin extracts ofvarious

RAW 264.7			
Compound name	**Conc. µg/ml**	**OD at 550nm**	**% Inhibition**
Control	0	0.801	0.00
Black Cumin extract	10	0.755	5.74
	20	0.701	12.48
	40	0.652	18.60
	80	0.613	23.47
	160	0.571	28.71
	320	0.52	35.08

Concentrations and its% inhibition on RAW 246.7 cells

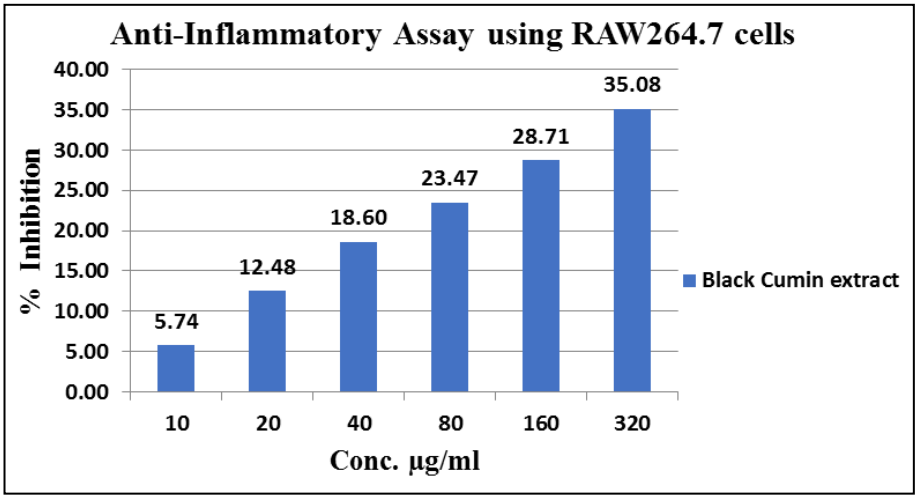


Fig 10: Effect of Black cumin extract on RAW 264.7 cells

Sample *Black Cumin extract* was tested for its activity on RAW264.7 cells against iNOS release post LPS stimulation. It is observed that, increase in concentration percentage of inhibition also increases gradually. Hence by these graph and table, Nigella sativa extract has significant level on anti-inflammatory activity.Some studies indicate that *Nigella sativa* could improve inflammation and reduce oxidative stress in patients with RA. It is suggested that *Nigella sativa* may be a beneficial adjunct therapy in this population of patients [15]. Some studies showed that N. sativa ethanol extract effectively inhibit histamine release from peritoneal Wistar rat mast cells proportionally to its concentration. N. sativa is effective as an anti-inflammation on mast cells by inhibition of histamine release and has no toxic effect on mast cell. N. sativa could be considered as a potential therapy for asthma therapy and prevention [16].

4. Conclusion

4.1 Anticancer: Cytotoxity studies for AGS and PANC-1 cell lines

Black cumin extract has shown the inhibition against both AGS and PANC – 1 cell lines with an IC50 value of 43.41µg/ml and 144.4µg/ml respectively. Here Black cumin extract hinders the cell growth by initiating Apoptosis. These results are important because they highlight the potential effects of *N. sativa* in the treatment of patients with cancer; thus encouraging researchers to conduct further studies in order to develop various and more effective formulations to treat an array of diseases, including cancer. Lately, *N. sativa* has become an important topic for research worldwide, but more studies need to be done to discover the different apoptotic mechanisms that further show the therapeutic efficiency of the plant against cancer.

4.2 Anti-inflammatory studies using RAW 264.7 cells

Black Cumin extract was tested for its activity on RAW264.7 cells against iNOS release post LPS stimulation. It is observed that the sample has shown up to 35.08% inhibition at highest concentration tested at 320µg/ml in RAW264.7 cells.

5. Acknowledgments

We thank Dr. A. P. Basavarajappa the principal, Dr. J Thimmashetty Professor, and Dr. Amit Kumar B Asst. Professor of Bapuji Pharmacy college, Davangere and Skanda

Life Science Pvt Ltd, Bengalore for providing there facilities which was very helpful for carrying out the project.

6. References

1. Ebrahim M, Yimer KaldBeshirTuem, Aman Karim, Najeeb Ur-Rehman, Farooq Anwar *et al*. *Nigella sativa* L. (Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses, Evid Based Complement, Alternat Med, 2019, 1528635.
2. Mohamed FR, Hassanien, Adel MA. Assiri and Hesham Farouk Oraby, Health-promoting value and food applications of black cumin essential oil: an overview, Journal of food science and technology. 2015: 52(10):6136-6142
3. Forouzanfar F, Bazzaz BS, Hosseinzadeh H. Black cumin (*Nigella sativa*) and its constituent (thymoquinone): a review on antimicrobial effects, Iranian Journal of Basic Medical Sciences, 01 Dec. 2014; 17(12):929-938.
4. Equar Taka, Elizabeth A Mazzio, Carl B. Goodman, Natalie Redmon, Hernan Flores-Rozas, Renee Reams, Selina Darling-Reed, and Karam F.A. Soliman. Anti-inflammatory effects of Thymoquinone in activated BV-2 microglia cells, HHS Author Manuscripts. 2015; 286:5-12.
5. Hamid Mollazadeh, Amir R Afshari, Hossein Hosseinzadeh. Review on the Potential Therapeutic Roles of *Nigella sativa* in the Treatment of Patients with Cancer: Involvement of Apoptosis-Black cumin and cancer, J Pharmacopuncture. 2017; 20(3):158-172.
6. Kourosh Hasanzadeh Ghahramanloo, Behnam Kamalidehghan, Hamid Akbari Javar, Riyanto Teguh Widodo, Keivan Majidzadeh, Mohamed Ibrahim Noordin *et al*. Comparative analysis of essential oil composition of Iranian and Indian *Nigella sativa* L. extracted using supercritical fluid extraction and solvent extraction. Drug Des DevelTher. 2017; 11:2221-2226.
7. Kausar H, Abidin L, Mujeeb M. Comparative Assessment of Extraction Methods and Quantitative Estimation of Thymoquinone in the Seeds of *Nigella sativa L* By HPL, International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(12):1425-1428
8. HammadShafiq, Asif Ahmad, Tariq Masud, Muhammad Kaleem. Cardio-protective and anti-cancer therapeutic potential of *Nigella sativa*, Iran J Basic Med Sci. 2014; 17(12):967-979.
9. Anna Czajkowska, Agnieszka Gornowicz, Natalia Pawłowska, Robert Czarnomysy, Jolanta Nazaruk, Wojciech Szymanowski *et al*. Anticancer Effect of a Novel Octahydropyrazino [2,1-a:5,4-a′]diisoquinoline Derivative and Its Synergistic Action with *Nigella sativa* in Human Gastric Cancer Cells,Biomed Res Int. 2017; 2017:9153403.
10. Prashanth Rawla, Adam Barsouk. Epidemiology of gastric cancer: global trends, risk factors and prevention, Prz Gastroenterol. 2019; 14(1):26-38.
11. M. Akram, Khanand M Afzal. Chemical composition of *Nigella sativa* Linn: Part 2 Recent advances, Inflammopharmacology. 2016; 24:67-79
12. Muhammad Jahangir Hossen, Woo Seok Yang, Daewon Kim, AdithanAravinthan, Jong-Hoon Kim, Jae Youl Cho *et al*. Thymoquinone: An IRAK1 inhibitor with *in vivo* and *in vitro* anti-inflammatory activities, Sci Rep. 2017; 7:42995.
13. Prashanth Rawla, Tagore Sunkara, Vinaya Gaduputi. Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors, World J Oncol. 2019; 10(1):10-27.
14. Amin F Majdalawieh, Muneera W. Fayyad, Recent advances on the anti-cancer properties of *Nigella sativa*, a widely used food additive, J Ayurveda Integr Med. 2016; 7(3):173-180.
15. VahidHadi, Sorayya Kheirouri, Mohammad Alizadeh, Alireza Khabbazi, Hossein Hosseini, 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 J Phytomed.2016; (1):34-43.
16. Mukhtar Ikhsan, NurulHiedayati, Kazutaka Maeyama, and FarizNurwidya, *Nigella sativa* as an Anti-inflammatory agent in asthma, BMC Res Notes, 2018; 11(1):744.
17. Mohammed SJ**,** Amin HHH, Aziz SB, Sha AM, Hassan S, Abdul Aziz JM. Structural Characterization, Antimicrobial Activity, and *In Vitro* Cytotoxicity Effect of Black Seed Oil, Evidence-based Complementary and Alternative Medicine: Ecam. 2019, 2019:6515671.