Pak. J. Biochem. Mol. Biol., 2022, 55(2):100-108

Research paper

Antioxidant and chemo-preventive potential of *Nigella sativa* on PSN-1 cell line in rats

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Abstract:

Nigella sativa is an important herbal plant, commonly known as black seed, and consumed throughout the world. Nigella is reported as antidiabetic, antihypertensive, anticancer, and analgesic. The present study was designed to evaluate the anticancer and antioxidant activities. Wister Albino rats (n=24) were divided into 4 groups. Animals were divided into 4 groups, Control was given normal saline, and the second group was given Nigella extract at a dose of 250 mg/kg/ml orally. In group 3, mercury at a dose of 0.3 mg/kg/ml was administered intraperitoneally and group-4 was pretreated with Nigella extract, and post 1-week administration of extract mercury was co-administered for 2 weeks. Cancer induced in Wistar albino male rats via initiation of oxidative stress through Mercuric chloride administration. The anticancer potential of Nigella was determined by an in vitro assay (MTT assay). Following antioxidant activity of Nigella was determined in rats. In both in vitro and in vivo studies, Nigella reduced cancer cells metabolism as well as oxidative stress respectively. Present studies reveal that induction of oxidative stress can initiate cancer which can counter Nigella administration by reducing oxidative stress and maintenance of plasma antioxidant enzyme levels, further it could be helpful as an anticancer activity.

Keywords: Nigella, Oxidative stress, Anticancer, Mercuric chloride

INTRODUCTION

Nigella sativa, commonly called black seeds belongs to the family Renunculacae and from many centuries it has been used as an ailment for different diseases. Among Muslims it is considered as the cure for every disease [1]. Researchers have shown that Nigella seeds possess a great pharmacological potential and a lot of biochemical activities such as immunomodulatory, analgesics, antimicrobial, anti-inflammatory, anti-spasm, bronchodilator, hepatoprotective and diuretics [2,3,4,5].

Cancer is considered as the most life threatening disease and a big life losing cause in the world. Its actual cause is still not cleared completely but most of the scientists said that it is due to changing of genetic pathways [6-10]. Cancer is defined as uncontrolled cell proliferation induced by a gene mutation. As a result, every anti-cancer treatment must therefore shield DNA from modification or destroy genetically modified cancer cells [11].

Liu and Lewis (2014) [12] reported that different industrial manufacturing and the content of different pollutants is getting increased day by day. Such pollutants are most commonly the heavy metals. Among all these metals, mercury pollution has adopted an important and extra care due to a fact that it may cause higher toxicity in living individuals and is also responsible for its inherent toxicity [13]. At initial level, a person may expose to Hg via food intake and inhalation and causes toxicities like neurotoxicity, hepatotoxicity, nephrotoxicity, and gastrointestinal toxicity accompanied with ulceration and hemorrhage [14]. It has been shown that the organic compound of mercury called methyl mercury, exhibits a great ability of penetration into the central nervous system which leads to neurological disorders [15,16]. Some studies revealed that ingestion of methylmercury is mainly due to sea food consumption, and it causes

various disorders and neurological problems [16,17,18,19,20]. Moreover, mercury causes calcium homeostasis imbalance, disrupting membrane potential, altering protein synthesis, and interrupting excitatory amino acid pathways in the central nervous system hence causing great loss to biochemical mechanisms [21]. Hence, the main aim of this study was to evaluate the preventive effects of Nigella following mercury administration in rats.

MATERIAL AND METHODS

We used 24 Albino Wistar rats in this study. All experiments were conducted according to GLP and ethical guidelines. Animals were assigned into four groups. Group 1 was Control and received only water and normal saline. Group 2 received 250 mg/kg/ml of NS extract orally for three weeks. Group 3 received only 0.3mg/ml/kg intraperitoneally Hg for two weeks. Group 4 pretreated with 250mg/kg NS extract for one week and then received 0.3mg/ml/kg Hg intraperitoneally for further two weeks with co administration on Nigella.

Chemo Preventive Effect of *Nigella sativa*

Preparation of Nigella Extract

Nigella sativa seeds were crushed to fine powder and 1x PBS was added in it (15gm Nigella powder in 20ml PBS). Centrifugation of this sample was done over 12000rpm for 20 minutes at the temperature of 4˚C. After centrifugation supernatant was collected and filtered by using 0.22µ syringe filter.

Gel Filtration Chromatography (GFC)

The Nigella seed extract was fractionated by GFC using HiLoad 16/600, Superdex 200 pg column with 40 ml void volume, pre-equilibrated with PBS elution buffer at a flow rate of 1ml/minute. The absorbance was measured at 280 nm.

Lyophilization

Peaks/fractions were lyophilized. Their concentration was found out by Nanodrop.

Cell Culture

PSN1 cell line preserved as aliquot while was thawed. These cells were grown properly according to standard protocol i.e. at temperature of 37 ˚C, and 5% CO_2 supply for 24 hours in an incubator. When these cells were grown fully they were used for MTT assay plating.

MTT Assay

Cell line PSN- 1 was obtained from Molecular Biobank PCMD, University of Karachi for anticancer activity. Cells were cultivated in CO_2 incubator at 37 ˚C. MTT assay was performed to evaluate the cytotoxicity of protein fractions obtained through gel filtration chromatography. Cells were placed in 96-wells plate in DMEM plus 10% FBS. After completion of the incubation period, protein fractions were added in the wells in triplicates and incubated for 48 hours. After observing them, MTT dye was added and incubated again for 4 hours. The absorbance was measured at 570 nm. Percentage inhibition of cell viability was calculated through this formula:

$$\% \text{ inhibition} = \frac{(\text{Untreated-Treated}) \times 100}{\text{Untreated}}$$

Antioxidant Activity

Estimation of lipid peroxidation (LPO)

Lipid peroxidation expressed as MDA levels in organisms which indicates oxidative status, because the ultimate product of lipid peroxidation is MDA levels. Results of LPO were expressed in micromoles of MDA/ml of plasma (36).

Catalase assay

Catalase assays were done according to the reported protocol (35). The solution contained 0.1 ml of biological sample, 1.0 ml of 0.01 M Phosphate buffer (pH 7.4) and 0.4 ml of H_2O_2 (0.2 M). The tubes were incubated at 37 °C for 90 s. Catalase activity was expressed as micromoles of H_2O_2 consumed/minute/ milliliter of plasma

Statistical analysis

Statistical analysis was done by two-way ANOVA using SPSS Version 20.0. Results are represented as mean ±SD. Post Hoc Analysis was made by Tukey's Test.

RESULTS

Gel Filtration Chromatography

NS peaks were eluted at different time intervals at 280 nm. Peak 1 eluted at time 43-54 minutes, peak 2 at 61-69 minutes then at 98-102, 107-122, 131-139 minutes respectively as shown in chromatogram Figure 1.

Cytotoxicity Assay

The application of different peaks of *Nigella sativa* seeds obtained via gel filtration chromatography for the treatment of PSN-1 cell line showed a cytotoxic effect on cancer cells (Fig. 1). The MTT results showed in figure 2, a distinctive reduction in the number of viable PSN-1 cells which were treated by Nigella peaks as compared to control i.e., peak 1 (p,9.617E-07), peak 2 (p,0.006), peak3 (p,0.084), peak 4 (p,4.21618E-050) peak 5 (p,0.0039), peak 6 (p,0.0046), peak 7 (p,0.0040). The MTT assay demonstrated that treatment with given Nigella peaks at concentration of 50 µg/ml had toxic effect on PSN-1 cells as compared to control cells. The given graph shows the toxic effect of these peaks as compared to untreated control and PBS-1X.

Antioxidant activity

Figure 3 shows the effect of Nigella effect on LPO levels in rats. One-way ANOVA estimated significant effect ($F=21.297$, $df=17$, $p<0.05$) on LPO levels. Oral administration of Nigella extract increases LPO levels showing improving antioxidant mechanism in rats.

Figure 4 shows the effect of Nigella effect on Catalase activity in rats. One-way ANOVA estimated significant effect ($F=51.332$, $df=17$, $p<0.01$) on Catalase levels. Oral administration of Nigella extract increases Catalase levels showing an effective treatment of extract against oxidative stress in rats.

DISCUSSION

Since thousands of years, humans use herbal medicines for curing diseases. The herbal medicines are supposed to use without any harmful effects as compared to synthetic drugs [22]. *Nigella sativa* possess has pharmacological potential and a lot of biochemical activities. The previous researches shows the useful effect of Nigella seeds against various diseases [1-5,23]. It is protective against cytotoxicity of different soft organs. In the present study Nigella extract was used to find its effect on $HgCl_2$ induced oxidative stress and on pancreatic ductal adenocarcinoma cell line (PSN-1) through MTT assay. All Protein fractions/peaks showed inhibition of cells viability as compared to control. The highest inhibition that was given by a fraction was highly significant i.e. 69%. Suggested mechanism of Nigella can be illustrated that essentially the active components thymoquinone (TQ) from N. sativa effects on cancer to assist them die via various biochemical mechanisms, including (1) TQ causes cell apoptosis in cancer cells by upregulating gene expression of apoptosis causing genes (caspases and bax) while down regulating gene expression in anti-apoptotic gene. (e.g., bcl2) [11]; (2) TQ inhibits Akt activation through dephosphorylation, thereby inhibiting cancerous cells existence [37]; (3) TQ causes deactivation of NF-kappa B pathway by causing cytokines to be produced, thereby controlling oncogenes from getting expressed [24] ; (4) TQ tends to increase antioxidants' enzyme activity and thereby protecting cells from cancer [25,26]; (5) TQ shields normal tissue against the damage induced by ionizing radiation in cancer therapy [27]; (6) TQ prevents CYP450 enzymes from damage [28].

Change of balance between the production of free radicals and reactive metabolites simply termed as oxidative stress. Their

inaccurate balance causes damage to important biomolecules and cells [29]. Reactive oxygen species formation occurs for a long time if there is some stress inside the body. It leads to the cellular destruction by inhibition of their function. These damages become a strong reason for the induction of mutations and neoplastic transformation [30]. Increase in DNA mutation and genome instability results in cancer initiation and ultimately its progression occurs [31]. so oxidative stress leads to cancer [32] therefore anticancer behavior of Nigella was further described through its antioxidant potential. This study also suggested that cancer can also be cured at very initial stage when these radicles are being synthesized inside the body. Antioxidant properties of Nigella in rats were shown by estimating Lipid peroxidase and catalase following mercury administration. Nigella treated group showed improved enzymatic level. Previous studies suggested that the treatment of Nigella caused significant decrease in the occurrence and multiplicity of cancers in the lungs and various regions of the intestine, notably the esophagus and frontal stomach [33]. According to several research, Nigella treatment has substantial anti - proliferative effects on rat cancer progression and cell growth in a variety of organs including colonic, lungs, esophagus, and frontal stomach cancers right after the initiation-phase [34].

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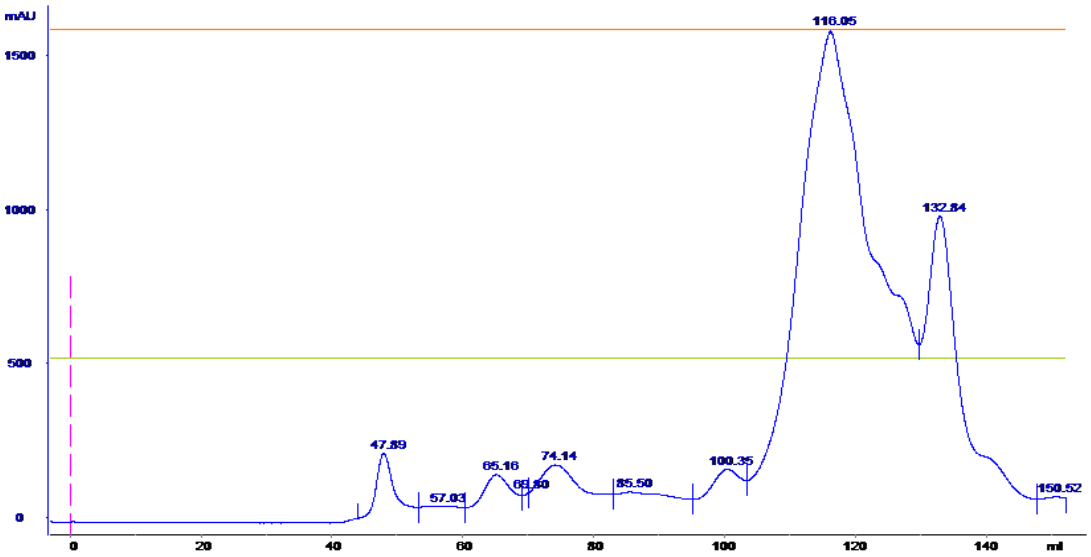

Figure 1: Gel Filtration Chromatogram

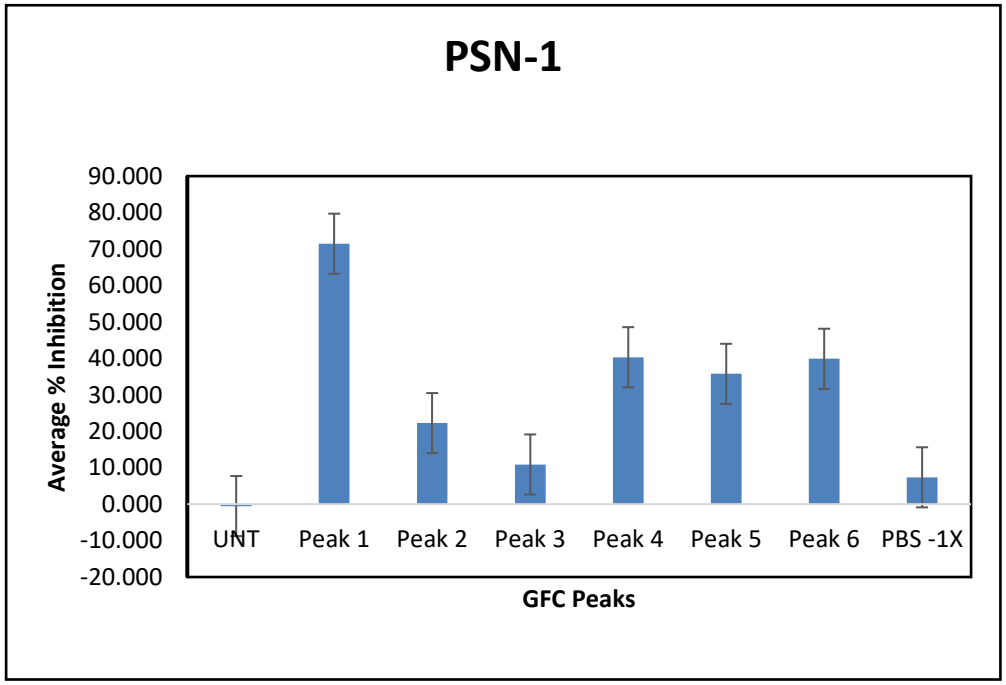

Figure 2: Cytotoxic effect of *Nigella sativa* on PSN-1 cells

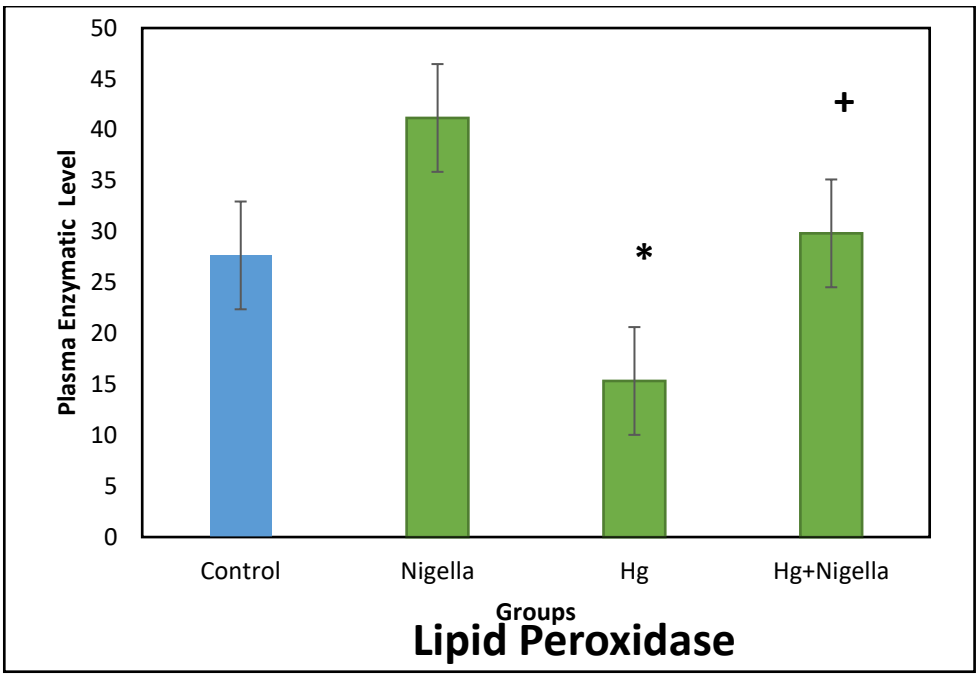

Figure 3: Effect of *Nigella sativa* and Mercury on plasma lipid peroxidase enzyme

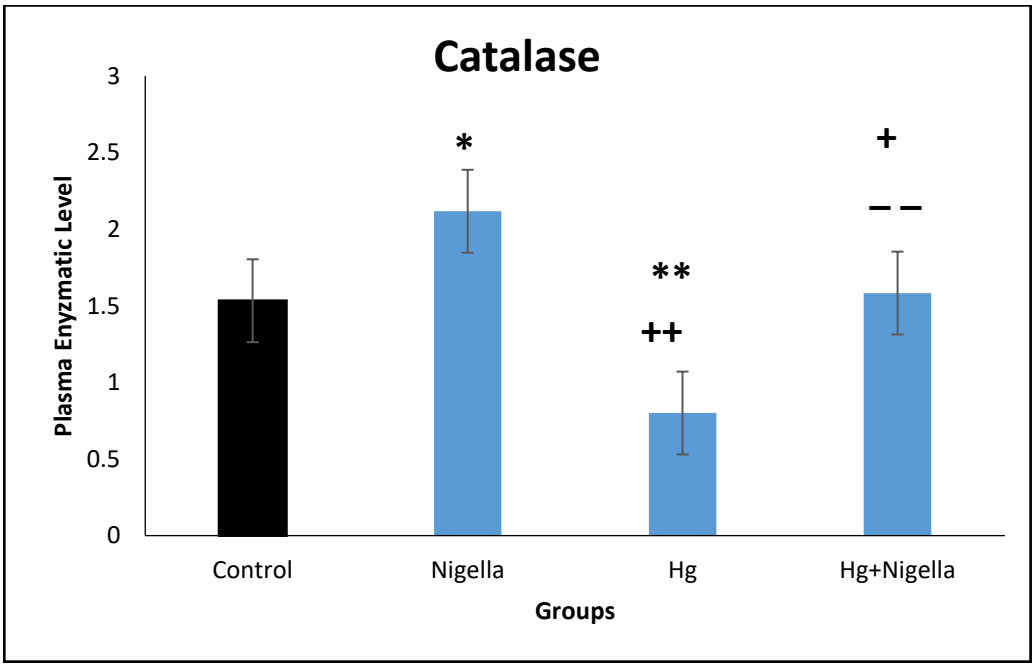

Figure 4: Effect of *Nigella sativa* and Mercury on plasma Catalase enzyme

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