Int J Pharm Bio Sci 2014 July; 5(3): (P) 472 - 477

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF ANTIOXIDANT PROPERTIES AT DIFFERENT GERMINATION STAGES OF SEEDS OF *NIGELLA SATIVA*

DR.DEVENDRA SINGH KUSHWAH*

Assistant professor, NSCB medical college ,Jabalpur(M.P.)

ABSTRACT

Among the promising medicinal plants, Nigella sativa, also known as Black seeds and Black cumin, has been called the “Blessed Seed” for its miraculous curing ability. Present study has been carried out to evaluate anti-oxidant properties at different germination stages of seeds of *Nigella sativa.* The level of antioxidant enzymes, SOD and CAT decreased gradually in the samples from first day to fourth day. The activity of SOD and CAT was significantly higher in seed sample as compared to first four days after the start of imbibition. Contrary to CAT and SOD activity, PEROXIDASE activity showed significant increase during germination till sixth day after which there was a decline till 8^{th} day of germination. we can conclude that activities of the antioxidative enzymes CAT and SOD may be involved in preserving the viability of seeds and protecting them from reactive oxygen species formed during storage and seed germination. Peroxidase activity may have a role in the early stages of development of *Nigella* seedlings. In order to further investigate the role of PEROXIDASE in the early and later stages of seedling development, this isoform of PEROXIDASE has to be analyzed by isolation and kinetic characterization.

KEY WORDS: ***Nigella sativa,*** **anti-oxidant property, catalase, SOD, Peroxidase**

DR.DEVENDRA SINGH KUSHWAH
Assistant professor, NSCB medical college ,Jabalpur(M.P.)

INTRODUCTION

Among the promising medicinal plants, Nigella sativa, also known as Black seeds and Black cumin, has been called the "Blessed Seed" for its miraculous curing ability. The results of extensive pharmacological studies justify the broad, traditional therapeutic value of Black Seeds. These studies found Black Seed to have analgesic[1] antilipemic[2, 3] post coital contraceptive[4], diuretic and antihypertensive[5], bronchodilator and calcium antagonist[6], histamine release inhibitor[7], hepatoprotective[8], anthelmintic[9], antifungal[10], antimicrobial (against a wide range of organisms) [11], anticancer[12], and anti-inflammatory activities[13]. *Nigella sativa* has been used since ancient times, in Asia, Middle East, and Africa, as analgesic, anti-inflammatory, antiallergic, antioxidants, anticancer, antiviral and for general well-being . In Islam, it is regarded as one of the greatest forms of healing medicine available. Traditionally, ROS were considered to be toxic by-products of aerobic metabolism. The toxic products of ROS such as hydrogen peroxide (H_2O_2) and superoxide (O_2 -) are produced in a number of cellular reactions, including the iron-catalysed Fenton reaction, and inhibition of enzymes such as lipoxygenases, peroxidases, NADPH oxidase and xanthine oxidase. ROS can cause oxidative damage to many cellular components, which are lipids (for example, peroxidation of unsaturated fatty acid in membranes), proteins (denaturation and oxidation), carbohydrates and nucleic acids. Oxidative damage may result from the alteration of the balance between the production of ROS and their detoxification by the antioxidative systems. According to the properties of ROS, plant cells require at least two different mechanisms to regulate their intracellular ROS concentrations by scavenging of ROS: one that will enable the fine modulation of low levels of ROS for signaling purposes, and one that will enable the detoxification of excess ROS, especially during stress conditions. To prevent oxidation of cellular components, cells maintain low, steady state levels of ROS by a variety of enzymatic and nonenzymatic antioxidant mechanisms. Major ROS scavenging in plants by a protective system of enzymes includes superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). The pathways of ROS-scavenging in plants, including SOD are found in almost all cellular compartments, the water cycle in chloroplasts, the ascorbate-glutathione cycle in chloroplasts, cytosol, mitochondria, apoplast and peroxisomes, glutathione peroxidase, and CAT in peroxisomes. The upstream enzyme SOD acts as the first line of defense system converting superoxide (O_2-) into hydrogen (H_2O_2) and dioxygen (O_2), to protect cells against the oxidative damages. Since superoxide radicals are found to be toxic to living cells, oxidizing and degrading biologically important molecules, the observations on SOD activity in different plant species suggest that different mechanisms may be involved in oxidative stress injury. The biological importance of SOD has been examined. This has mainly been done in relation.

AIMS AND OBJECTIVES

1. To standardize the germination of *Nigella sativa* seeds under normal and stress conditions.
2. To study the morphological changes during each germination stage under normal and cadmium stress conditions.
3. To assay antioxidant enzymes (SOD, CAT, APX & PEROXIDASE) in each germination stage.
4. To assess hydroxyl free radical scavenging activity and lipid peroxidation in each germination stage.
5. To assay antioxidant enzymes (CAT & PEROXIDASE) in each germination stage under cadmium stress.
6. To assess hydroxyl free radical scavenging activity and lipid peroxidation in each germination stage under cadmium stress.

MATERIALS AND METHODS

3.1 COLLECTION OF Nigella sativa SEEDS

Seeds of *N. sativa* were procured in December, 2009 from a local grocery store in Lucknow, India and surface sterilized with 1%

HgCl for 30 min. They were rinsed with tap water followed by double distilled water and allowed to soak in de-ionized water and 2mM solution of cadmium chloride.

3.2 GERMINATION OF *Nigella sativa* SEEDS

Nigella sativa seeds were selected for the study of its antioxidant activities during different stages of germination. Seeds of *Nigella sativa* were grown in glass petri plates having two or three folds of damp blotting paper in distilled water at room temperature of about 32^0C. 0.25gm seeds were inoculated in each petri plate under aseptic condition. The complete germination took eleven days with epicotyl, hypocotyl, root and green leaves. The seeds were incubated in dark till sprouting was initiated (3 days) after which the plates were transfered to culture room at a light intensity of 100 µmol m^{-2} $s^{-1}$and a 14/10 h (day/night) photoperiod till the complete plantlet with two leaves were obtained.

Germination under cadmium stress

Seeds of *Nigella sativa* were grown in glass petri plates heaving two or three folds of blotting paper in 2mM cadmium chloride solution. 0.25gm seeds in each petri plate were grown till 11days. Antioxidant activity was also analyzed in different seed germination stages in the presence of cadmium stress.

- ***Study of germination stages of Nigella sativa seeds***

Morphological changes during seed germination were studied. During different germination stages from1 to 11 days studied, time of sprouting initiation ,length of sprout , emergence of hypocotyls, epicotyls, root and their length. Morphological characters were studied in different germination stages and the dry and the fresh weight of the samples of each day was taken.

- ***Harvest of germinated seeds***

The antioxidant activity was analyzed using different germination stages *Nigella sativa* seeds. Forceps were used to pick out seeds from plate, seeds were put on blotting seat to adsorbed water. The seeds collected for different experiments were used immediately for preparing enzyme extracts. Three replicates petri dishes were prepared from each treatment (0.25g per petri dish). Seeds were considered to be germinated after the radical emerged from the testa. All the samples were stored at -80^oC in a deep freezer until used further. Biochemical evaluations were performed to determine the activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidases (PEROXIDASE). The nitric oxide (NO) free radical scavenging activity of the extracts was measured and lipid peroxidation (LPO) was also evaluated.

- ***Statistical Analysis***

Every experiment was repeated thrice and all the results were expressed as mean value ±SD for three replications. For each replication plant material was taken by weight from different stages of germination.

- ***Preparation of extracts***

Different enzymes were assayed in each germinating stage of the seed. For preparation of crude extract, 0.25 gram of plant material was homogenized in chilled mortar and pestle with ice cold 5 ml of 50mM phosphate buffer (pH-7.8). Homogenate were centrifuges for 10 min at 10,000 rpm at4°C and supernatant was collected directly used for assay of SOD, CAT, APX and GPX, NO and LPO as described by respectively. Enzyme activities were referred to the sample fresh weight.

- Estimation of SUPEROXIDE DISMUTASE was done by Gianopolitis and Ries -1977 method.
- Estimation of CATALASE was done by Aebi*,* 1984 method.
- Estimation of LIPID PEROXIDATION was done by Ohkawa *et al.method.*

RESULTS

DAY	Activity (U/mg) during various phases of germination of *Nigella sativa* in $CdCl_2$ stress		
	CAT	SOD	LIPID PEROXIDATION (%)
0	3716.30±0.41	16.28±0.29	91.78±0.38
1	1651.40±0.35	12.25±0.36	83.31±0.81
2	1652.60±0.31	10.66±0.23	76.90±0.64
3	1651.50±0.25	12.24±0.34	53.20±0.35
4	2478.10±0.24	10.29±0.43	46.02±0.34
5	3302.50±0.31	12.19±0.27	43.30±0.42
6	2891.20±0.36	17.88±0.16	40.64±0.79
7	3714.70±0.25	16.21±0.36	56.61±0.73
8	5366.20±0.41	43.06±0.66	61.82±0.61
9	7027.60±0.34	65.68±0.44	67.00±0.34
10	7844.20±0.39	75.36±0.50	70.93±0.31
11	7432.00±0.28	80.88±0.45	71.06±0.62

Superoxide dismutase and Catalase

The level of antioxidant enzymes, SOD and CAT decreased gradually in the samples from first day to fourth day. The activity of SOD and CAT was significantly higher in the seed sample as compared to first four days after the start of imbibition. The activity of these two enzymes was seen to increase from the fifth day to the tenth day. The complete seedling was formed on the eleventh day of germination and the activities of SOD and CAT was lesser in seedling as compared to tenth day of germination. This result is in compliance with the research of scientists and published data. SOD and catalase decreased gradually during the first five days of germination. The activity of these enzymes was significantly higher in 0.05% carbendazim treated seeds compare to control seeds ($p>0.01$). The decrease in the levels of SOD and CAT in the presence of 0.1% and 0.3% carbendazim might be related to the higher use of these antioxidants to fight the ROS produced enormously during stress. Another research which supports the above result is shown by another group of researchers in *P. omorika seeds*. As no changes in enzyme activity were detected in *P. omorika* seeds up to 4th day after the start of imbibition, we here present specific activity of the enzymes catalase, superoxide dismutase, and peroxidase from the 4th day, when most of the seeds germinated. nder cadmium stress, it was seen that CAT activity showed an almost similar pattern as in seeds germinated under normal conditions. The activity was constant till the 3rd day after which it increased till the 10th day of germination. A slight decline was noticed on the 6th day of germination. The activity decreased in seedling as was also seen in normal germination.

Lipid Peroxidase

Contrary to CAT and SOD activity, PEROXIDASE activity showed a significant increase during germination till the sixth day after which there was a decline till the 8th day of germination. However, it was seen that the activity was slightly increased the 9th day of germination after the start of imbibitions till the formation of complete seedlings (11th day). In dry seeds also there was no PEROXIDASE activity seen. The specific activity of PEROXIDASE per fresh weight increased continuously till the 7th day and was highest on the 10th and 11th day of germination. The results indicate that, in wheat seeds**,** imbibition and germination are associated with enhanced cellular capacity to detoxify H_2O_2. For this detoxification the operation of ascorbate peroxidase together with the ascorbate-regenerating enzymes appears to be of particular importance. PEROXIDASE activity under cadmium stress showed more or less similar pattern as in normal (control) germination. Uniform trend was observed till the 7th day of germination after which it increased from 8th day to the 11th day. There was an abrupt increase from the 9th day of germination till the formation of seedling. It was seen ti decline slightly on the 7th day of germination after then there was sharp increase in PEROXIDASE activity till the formation of plantlet (11th day). The overall

pattern shows that during germination, PEROXIDASE activity was seen to increase as the germination proceeded under the stress of cadmium. In other words, it can be said that PEROXIDASE activity was less during earlier stages of germination whereas it was more during later stages.

Lipid peoxidation in normal germination and under cadmium stress

The percentage inhibition of hydroxyl free radical scavenging activity was seen to be more or less similar during the germination period till the formation of seedling. Maximum inhibition was observed on the 2nd day of imbibitions and least activity was seen on the 4th day. Inhibition was also seen to increase in the plantlet after complete germination. Under cadmium stress, percentage inhibition was seen to decrease from the 1st day to the 6th day after which it was increased from the 7th day to the 11th day till the formation of seedling. If we assess overall activity of these antioxidant enzymes when expressed in terms of its fresh weight it was seen that there was decreased CAT activity under cadmium stress when compared with germination under normal contions. The activity pattern of PEROXIDASE showed very interesting results in which the overall decrease was observed as compared to control germination but the activity under stress was much more during the 9th, 10th and 11th day of germination. The percentage inhibition of hydroxyl free radicals in terms of lipid peroxidation was very high in seeds which germinated under stress of cadmium ions during the whole germination process in each stage.

4.3 DISCUSSION

Catalase activity was increased in germinated seeds after 4 days of imbibition. This observation suggests that CAT activity in seeds and seedlings may be involved in preservation of viability during storage and also necessary for seed germination and early seedling growth. This is in accordance with previous results indicating that activity of antioxidative enzymes such as catalase is closely related with storage longevity and germination percentage of bitter gourd seeds (Yeh *et al*., 2005). Activity of SOD also showed the same pattern in terms of activity as catalase. However, its presence in all samples suggests that this enzyme may participate in protection against free superoxide radicals. Peroxidase activity showed the most notable changes during germination. Dry seeds exhibited showed no PEROXIDASE activity, but during germination this activity appeared and dramatically increased. From this fact it could be concluded that PEROXIDASE activity may have a role in the later stages of germination and in seedling development, but not in preservation of dry seeds. Peroxidase activity was not detected even in imbibed seeds before the start of germination in tomato (Mo r o h a s h i, 2002) and *Chenoperoxidaseium rubrum* (D u č i ć et al., 2003/4; Mi t r o v i ć et al., 2005).

4.4 CONCLUSION

In summary, we can conclude that the activities of the antioxidative enzymes CAT and SOD may be involved in preserving the viability of seeds and protecting them from reactive oxygen species formed during storage and seed germination. Catalase activity in seeds can serve as a parameter that indicates the germination capacity of dry seeds. Peroxidase activity may have a role in the early stages of development of *Nigella* seedlings. In order to further investigate the role of PEROXIDASE in the early and later stages of seedling development, this isoform of PEROXIDASE has to be analyzed by isolation and kinetic characterization.

REFERENCES

1. Abdel-Fattah A M, Matsumoto K, Watanabe H. Antinociceptive effects of Nigella sativa oil and its major component, thymoquinone, in mice. Eur J Pharmacol. 2000; 400:89–97.
2. Bashandy SA. Effects of Nigella sativa oil on liver and kidney functions of adult and

senile rats.Egypt J Pharm Sci. 1996; 37:313–27.
3. Hassanim NI, Hassan FM. A preliminary study on the effect of Nigella sativa seeds on hypoglycemia. Vet Med J Giza. 1996; 44:699–708.
4. Keshri G, Singh MM, Lakshmi V, Kamboj VP. Post-coital contraceptive efficacy of the seeds of Nigella sativa in rats. Indian J Physiol Pharmacol. 1995; 39:59–62.
5. Zaoui A, Cherrah Y, Lacaille-Dubois MA, Settaf A, Amarouch H, Hassar M. Diuretic and hypotensive effects of Nigella sativa in the spontaneously hypertensive rat. Therapie. 2000; 55:379–82.
6. Gilani AH, Aziz N, Khurram IM, Chaudhary KS, Iqbal A. Bronchodilator, spasmolytic and calcium anatagonist activities of Nigella sativa seeds (Kalonji): A traditional herbal product with multiple medicinal uses. J Pak Med Assoc. 2001; 51:115–20.
7. Chakravarty N. Inhibition of histamine release from mast cells by nigellone. Ann Allergy.1993; 70:237–42.
8. Daba MH, Abdel-Rahman MS. Hepatoprotective activity of hymoquinone in isolated rat hepatocytes. Toxicol Let. 1998; 95:23–9.
9. Akhtar MS, Riffat S. Field trial of Saussurea lappa roots against nematodes and Nigella sativa seeds against cestodes in children. J Pakistan Med Assoc. 1991; 41:185–7.
10. Khan MA, Ashfaq MK, Zuberi HS, Mahmood MS, Gilani AH. The in vivo antifungal activity of the aqueous extract from Nigella sativa seeds. Phytother Res. 2003; 17:183–6.
11. Sokmen A, Jones BM, Erturk M. The in vitro antibacterial activity of Turkish medicinal plants. J Ethnopharmacol. 1999; 67:79–86.
12. Worthen DR, Ghosheh OA, Crooks PA. The in vitro anti-tumour activity of some crude and purified components of black seeds, Nigella sativa L. Anticancer Res. 1998; 18:1527–32.
13. Mutabagani A, El-Mahdy , Samiha HM. A study of anti-inflammatory activity of Nigella sativa L and thymoquinone in rats. Saudi Pharm J. 1997; 5:110–3.
14. Giannopolitis CN and Ries SK (1977) Superoxide dismutases. 1. occurrence in higher plants. Plant Physiology 59: 309-314
15. Aebi H (1984). "Catalase in vitro". Meth. Enzymol. 105: 121–6. doi:10.1016/S0076 6879(84)05016-3. PMID 6727660.
16. Ohkawa, H.,Ohishi, Í. and Yagi, Ê. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction; Anal. Biochem., 95: 351-358
17. Yeh Y. M., Chiu K. Y., Chen C. L., and J. M. Sung (2005). Partial vacuum extends the longevity of primed bitter gourd seeds by enhancing their anti-oxidative activities during storage. Sci. Horti. 104, 101-112.
18. Dučić, T., Lirić-Rajlić, I., Mitrović, A., and K. Radotić (2003). Activities of antioxidant systems during germination of Chenopodium rubrum seeds. Biol. Plant. 47, 527-533.