Protective effect of black seed oil of *Nigella sativa* in rats during tumour induction and radiotherapy

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Abstract

The present study was conducted to evaluate the potency of *Nigella sativa* freshly crushed seeds (0.42 g/ kg body weight) or oil (2.5 ml/kg body weight) for preventing tumor induction through exposure of rats to a common pollutant (1,4-Dioxane) as a promoter under condition of the presence of an initiator (N-nitrosodiethylamine). The antitumor effect was evaluated alone or in combination with low doses of irradiation as a route of cancer treatment.

Female Swiss albino rats were administered orally twice weekly with *Nigella sativa* before and during exposure of rats to the carcinogenic compounds. Animals were exposed to 3 doses of radiation (3 Gy/ dose) day after day 2 weeks before the end of the experiment. The animals were scarified after one week of radiation. Homocysteine, glutathione, lipid peroxide, GGT activity, nitric oxide, total protein, albumin and billirubin levels were estimated in blood after 7 and 12 months from the start of the experiment. This work also includes histopathological study. Rats injected with the carcinogenic compounds showed marked elevation in homocysteine, GGT activity, nitric oxide, billirubin and lipid peroxide levels accompanied by a significant decrease in glutathione, total proteins and albumin levels. Pretreatment with *Nigella sativa*

alone or combined with γ- irradiation potentially reversed the investigated parameters.

As a conclusion, *Nigella sativa* significantly suppressed the growth of the tumor and efficiently produced synergistic effect with γ-irradiation. Therefore, *Nigella sativa* may be a good candidate to prevent tumor induction and so, it is advicable to use freshly crushed seeds during irradiation treatment in cancer patients as they gave more pronounced protection than the oil extract.

Aim of the work

The present study aims to evaluate the effect of promising natural product compound *(Nigella sativa)* for preventing tumour induction through exposure the rats to a common pollutant (1, 4 Dioxane) under condition of the presence of an initiator (N-nitrosodiethylamine). In addition study the synergistic effects of radiation exposure as a route of cancer therapy and treatment by freshly crushed seeds of *Nigella sativa* or the oil extract.

CONTENTS

Introduction

1,4-Dioxane is an air pollutant. Exposure to 1,4-dioxane may occur during its manufacture and its use as a solvent in a wide range of organic products and it has been detected in ambient air. 1,4-Dioxane is classified as a probable human carcinogen **(IARC, 1999)**. 1,4-Dioxane was shown to act as a tumour promoter in rat liver and mouse skin carcinogenisity assays. 1,4-dioxane potential cancer risk causes liver and nasal cavity tumours **(Stickney *et al.*, 2003).**

The liver tumours were seen at 1,4-dioxane drinking water concentrations of 0.05% for mice and 0.1% for rats. The nasal tumours in rats were observed at 1,4-dioxane drinking water concentrations of 0.5% **(Miyagawa *et al.*, 1999)**.

The liver tumours are considered to be associated with cytotoxicity and organ damage, which seem to occur in particular at dose levels at which 1,4-dioxane metabolism becomes saturated **(European Commission, 2002).**

N-nitrosodiethylamine is documented as a carcinogen that acts directly on the liver **(Ha *et al.*, 2001).** Also, it is used to initiate hepatocarcinogenesis (**Espandiari *et al.*, 2005).**

The seeds of *Nigella sativa* Linn. *(Ranunculaceae),* commonly known as black seed or black cumin, are used in folk (herbal) medicine all over the world for the treatment and

prevention of a number of diseases **(Thabrew *et al.*, 2005).**

The seeds contain both fixed and essential oils, proteins, alkaloids and saponins. Much of the biological activity of the seeds has been shown to be due to thymoquinone, the major component of the essential oil, but which is also present in the fixed oil (**Muhammad *et al.*, 2002**).

The seeds are characterized by a very low degree of toxicity. Administration of either the seed extract or its oil has been shown not to induce significant adverse effects on liver or kidney functions. It would appear that the beneficial effects of the use of the seeds and thymoquinone might be related to their cytoprotective effect **(Ali and Blunden, 2003).** The seeds/oil have antifungal effect **(Khan *et al.*, 2003)**, anti-parasitic effect **(Mahmoud *et al.*, 2002)**, anti-inflammatory and analgesic effect **(Enomoto *et al.*, 2001)**, antihypertensive activities **(Zaoui et al., 2000**), hypoglycemic effect **(Fararh *et al.*, 2002)**, antitumour effect **(Salim and Fukushima, 2003)**, antioxidant effect **(Badary *et al.*, 2003)** and antinociceptive effects **(Abdel-Fattah *et al.*, 2000).** The black seed oil is used in the treatment of allergic diseases **(Kalus *et al.*, 2003)**. *Nigella sativa* has effects on the immune system **(Islam *et al.*, 2004**), gastro- intestinal tract **(EL-Abhar *et al.*, 2003)**, cardio vascular system **(Zaoui *et al.*, 2002)**, blood **(AL-Jishi and Abuo Hozaifa, 2003),** Genito-Urinary tract **(EL-Tahir *et al.*,**

1999) and on the respiratory system **(Gilani *et al.,* 2001).**

Chemical composition, including active principles, of *Nigella sativa* (Muhammad et al., 2002)

	Sub-group	Components
Fixed oil (32-40 %)*	Unsaturated fatty acids	Arachidonic, eicosadienoic, linoleic, linolenic, oleic and almitoleic acid
	Saturated fatty acids	Palmitic, stearic and myristic acid
		Beta-sitosterol, cycloeucalenol, cycloartenol, sterol esters and sterol glucosides
Volatile oil (0.4-0.45 %)*		Nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, α & β-pinene, d-limonene, d-citronellol, p-cymene and 2–(2–methoxypropyl)–5–methyl–1,4–benzenediol
Proteins (16-19.9 %)*	Amino acids	Ala, Val, Arg, Asp, Gly, Ile, eu,Lys, Met, Phe, Pro, Ser, Thr, Tyr, Glu.
Alkaloids		Nigellicine, nigellidine, nigellimine-N-oxide
Coumarins		6–methoxy–coumarin 7–hydroxy–coumarin 7–oxy–coumarin
Saponins:	Triterpenes	Alpha-hedrin
	Steroidal	Steryl-glucosides, acetyl-steryl-glucoside
Minerals (1.79-3.74 %)*		Calcium, phosphorous, potassium, sodium and iron
Carbohydrates (33.9%)* Fiber (5.5 %)* water (6 %)*		

Results

Total plasma homocysteine

Total plasma homocysteine level showed a non significant change in the groups of oil extract (G2), crushed seeds (G3), oil extract+ irradiation (G4) and crushed seeds+ irradiation (G5) groups under investigation compared to the control (G1) group after 7 and 12 months.

Total plasma homocysteine level recorded a highly significant increase in the dioxane (G6) group ($P<0.001$) compared to the control (G1) group with % change +22.9 after 12 **months of treatment**.

Total plasma homocysteine concentration recorded a highly significant decrease in the group of dioxane + oil extract (G7) ($P<0.01$) compared to the dioxane (G6) group with % change -12.4 after 7 months.

Total plasma homocysteine concentration recorded a significant decrease in the groups of dioxane + crushed seeds (G8), dioxane + irradiation (G9) and dioxane + oil extract+ irradiation (G10) groups ($P<0.01$) compared to the the dioxane (G6) group with % change -11.8, -13.4, and -10.6 respectively after 12 months (Table (1) & Figs. 3a , 3b)