BJP

Bangladesh Journal of Pharmacology

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A Journal of the Bangladesh Pharmacological Society (BDPS); www.bdps.info
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
ISSN: 1991-0088

Bangladesh J Pharmacol 2014; 9: 15-21

Clinical Trial

A double-blind, randomize, placebo-control trial to evaluate the effect of *Nigella sativa* on palmar arsenical keratosis patients

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Article Info

Received: 11 December 2013
Accepted: 17 January 2014
Available Online: 20 January 2014

DOI: 10.3329/bjp.v9i1.17167

Cite this article:
Bashar T, Misbahuddin M, Hossain MA. A double-blind, randomize, placebo-control trial to evaluate the effect of *Nigella sativa* on palmar arsenical keratosis patients. Bangladesh J Pharmacol. 2014; 9: 15-21.

Abstract

This study was done to examine the role of *Nigella sativa* in 28 patients of palmar arsenical keratosis. Patients were randomized into two groups: Group A (n=15) receive capsules of placebo and vitamin E (200 mg) whereas Group B (n=13) receive capsules of *N. sativa* oil (500 mg) and vitamin E (200 mg) orally for 8 weeks. The mean (± SD) clinical scoring of palmar arsenical keratosis in Group A before and after treatment was 99.3 ± 21.5 and 62.3 ± 14.3 respectively (37% reduction). On the other hand, in *N. sativa* oil treated group, the mean clinical score was 119.1 ± 20.3 before treatment which was reduced to 39.2 ± 8.4 ($p<0.0001$) after treatment (67% reduction). There were 65% reduction of total arsenic in nail of Group A and 30% in Group B. In conclusion, oral administration of *N. sativa* oil improves the symptom of palmar arsenical keratosis as a result of reduction of body arsenic load.

Introduction

Arsenical keratosis is an important skin manifestation of chronic consumption of arsenic through drinking water. It appears either in both palms and/or soles. Presence of keratosis in palm is a social problem as it causes social stigma. In case of young unmarried girl it may even causes marriage related problems.

There is no effective treatment for palmar arsenical kera -tosis still now. Soothing effect following topical applica -tion of salicylic acid (Islam et al., 2007) or propylene glycol (Dina and Misbahuddin, 2010) suggests the use in palmar arsenical keratosis. On the other hand, some of our foodstuffs have the capability to remove arsenic from the body. These include beta-carotene, retinol (Hall, 1946), spirulina (Fariduddin et al., 2001; Misbahuddin et al., 2006), spinach (Umar, 2007), corn (Chowdhury et al., 2009), etc. However, cessation of these drugs often recur the symptoms. These treatments may affect on patient's adherence.

Nigella sativa (Kala jerra) is an annual flowering plant belongs to the family Ranunculaceae. Its seeds used widely in all levels and religions in Asia, Middle East and Africa as herbal medicine, in religious purpose and for cooking. Because of possessing immense healing power, in Islam, theses seeds are regarded as a predic=- tive medicine. These seeds are also used as immune- modulatory (Salem, 2005), antimetastatic (Al-Jishi and Abuo Hozaifa, 2003), regulation of leukocyte phagocyte -sis (Haq et al., 1995), regulation of lipid peroxidation (Hosseinzadeh et al., 2007), antiasthmatic (Kanter, 2009; Boskabady et al., 2010) effects. *N. sativa* seeds are a good source of oil (essential oil and fixed oil), flavonoids, saponins, proteins, alkaloids, carbohydrates and mine- rals like potassium, phosphorus, sodium and iron while zinc, calcium, magnesium, manganese and copper were found at lower levels (Burits and Bucar, 2000; Al-Jassir, 1992). Though thymoquinone, superoxide anion scaven -ger is the main active element of the volatile oil, extracted from *N. sativa* seed (Burits and Bucar, 2000), they also contain dithymoquinone, thymohydro-

quinone and thymol (Hosseinzadeh and Parvardeh, 2004). Experimental study revealed that *N. sativa* adsorbs cationic metals from waste water (El-Said et al., 2009). But up till now no such study has been carried out to observe the effectiveness of *N. sativa* on arsenicosis patients. Therefore, this present study was carried out in an attempt to investigate the effect of *N. sativa* in the treatment of palmar arsenical keratosis.

Materials and Methods

Study area

This double blind, randomized, placebo controlled trial was conducted in an arsenic-endemic area (Hotatia Union of Ramganj Upazilla, Lakshmipur District in Chittagong division), about 135 km away from the capital Dhaka city from February 2013 to April 2013. Total area of Ramganj Upazilla is 169.31 sq km with a population of 2,38,333 (male : female was 48: 52), using 41,930 tube wells for various purpose. Out of these 89.3% tube wells are contaminated with high concentration of arsenic (> 50 µg/L).

Study population

Sixteen thousand patients were registered in the record book of Ramganj Upazilla Health Complex. Out of these, 100 patients were selected randomly from Hotatia Union by online random number generator. Among them, 67 patients were found to suffer from mild to moderate degree of palmar arsenical keratosis. The clinical diagnosis of palmar arsenical keratosis was confirmed by a dermatologist. Lastly palmar arsenical keratosis was established by the existence of keratosis on palms, high concentration of arsenic in drinking water (>50 µg/L) and nail (>1 µg/g of nail). After counseling only 36 patients agreed to participate in this study. They were randomized two groups: Group A (received capsules of placebo and vitamin E) and Group B (received capsules of *N. sativa* oil and vitamin E). Though, the study was in progress with 18 patients with moderate palmar arsenical keratosis in each group, finally the study was completed with 15 patients in Group A and 13 patients in Group B (Figure 1). Total eight patients were dropped out from the study.

Both male and female participants with moderate palmar arsenical keratosis, age 20-65 years, having history of taking arsenic contaminated water (>50 µg/L) more than six months were enrolled for the study. However, patients receiving treatment of arsenicosis for last three months, suffering from chronic diseases like eczema, psoriasis, tuberculosis etc, patients with hepatic or renal failure, even pregnant or lactating mother or subjects who did not voluntarily agree to participate were excluded.

It is a single center trial. Each participant visited the Arsenic Camp (house of a patient) every two weeks interval for 8 weeks to collect the drugs, checking the adherence (by counting the remaining capsules in the packets), receiving sheets for monitoring adherence and adverse effects Sometimes patients were monitored randomly by direct contact over mobile phone.

Medication

During drug allocation a double blind method was

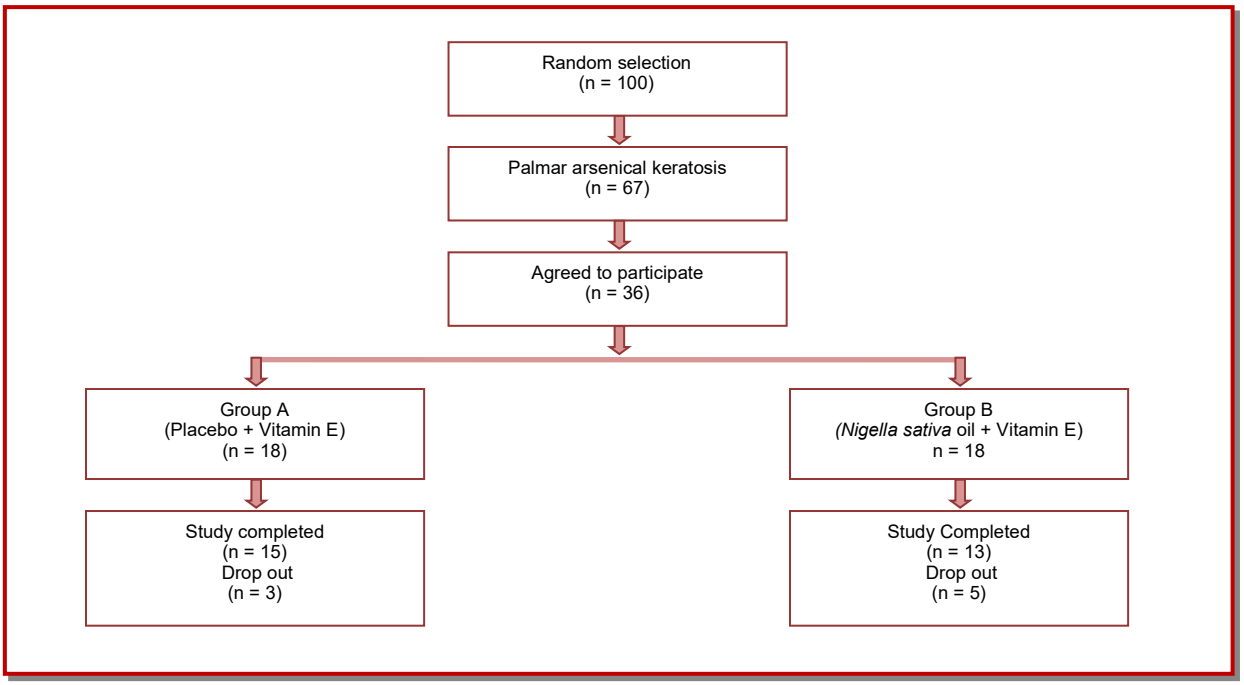

Figure 1: Flowchart showing subject selection and study design

Table I

Selected characteristics of the participants

Characteristics	Group A (Placebo + vitamin E)	Group B (*Nigella sativa* oil + vitamin E)
Number of participants (n)	15	13
Sex		
Male	5	2
Female	10	11
Age (Years)	46.8 ± 11.5	38.8 ± 12.8
Duration of arsenic exposure (Years)	18.4 ± 6.2	20.6 ± 7.3
Amount of arsenic in water (µg/L)	856.9 ± 324.9	826.6 ± 231.0
Duration of symptoms (Years)	12.6 ± 5.2	13.4 ± 6.8

Data were expressed as mean ± SD

followed by an assistant doctor, who did not know about the content of the container. The placebo and *N. sativa* oil was dispensed in capsule form with similar size, color and in envelops with similar labeling. Each patient received two envelops in each visit. One envelop contains 30 capsules of either *N. sativa* oil (500 mg) or placebo (refined oil with same size and color) and other envelop contains 15 capsules of vitamin E (200 mg). Patients of Group A received soft capsules of placebo twice daily and vitamin E once daily and Group B also received *N. sativa* twice daily and vitamin E once daily for 8 weeks without any interruption. Each patient was advised to swallow capsules daily with sufficient amount of drinking water. Patients tick marked daily the drug intake sheets and sheets of adverse effect(s) if any by themselves. However, all drugs of the study were prepared by a local pharmaceutical company (Drug International Limited).

Sample collection and estimation of parameters

Water, nails and blood were collected from all participants. Before starting the study, 50 mL of drinking water was collected from the tube well in a polypropylene container containing one drop of concentrated nitric acid to confirm the diagnosis. About one gram nails were collected in a small polyvenyl bag twice (before and after completion of treatment) to detect total amount of arsenic in the body. On the other hand, photographs of both hands were collected twice (before and after treatment) to monitor improvement of palmar arsenical keratosis. Moderate keratosis was palpable and visible affecting palm. In each patient, scoring of palmar arsenical keratosis was done every two weeks interval for 8 weeks by counting the number and measuring the size of the keratotic lesions in both palms by slide caliper. If the lesion size was <1 mm (as measured by slide caliper), the score was one. If it was in between 1 to 2 mm, the score is 2. If it was more than 2, the score was 3.

With all aseptic precaution 3 mL blood was collected twice (before and after treatment) in K_3EDTA containing test tube. Plasma was separated by ultra centrifugation (1,370× g for 10 min) and stored with labeled eppendorf at -10°C. Plasma glucose level was estimated by oxidase and peroxidase method, cholesterol and HDL-C (High Density Lipoprotein – Cholesterol) by CHOD – PAP method and triglyceride by GPO – PAP method using kits.

Nails were washed with acetone for 2 min and then dried before weighed. Total amount of arsenic in nail was estimated by silver diethyldithiocarbamate (SDDC) method using spectrophotometer at 525 nm wave length (Misbahuddin et al., 2006). Atomic florescence spectrophotometer was used to estimate the amount of arsenic in tube well water. Nails and water were digested by four acids (hydrochloric acid, nitric acid, sulfuric acid and perchloric acid).

Statistical analysis

One-way analysis of variance (ANOVA) was done for comparisons among groups and paired 't'-test was used for comparisons before and after treatment. The data were expressed as mean ± SD.

Results

The male female ratio was 1:2 in Group A and 1:5.5 in Group B (Table I). The mean (± SD) age was 46.8 ± 11.5 years in Group A and 38.8 ± 12.8 years in Group B. Out of 36 patients enrolled in the study, 28 patients completed the study protocol till week 8. Among the eight dropout cases, three were from Group A (did not attend the clinic) and five from Group B (one died of road traffic accident, two refused to give biological samples after completion of treatment, one due to pregnancy, one patient did not attend the clinic).

The mean duration of arsenic exposure in Group A was

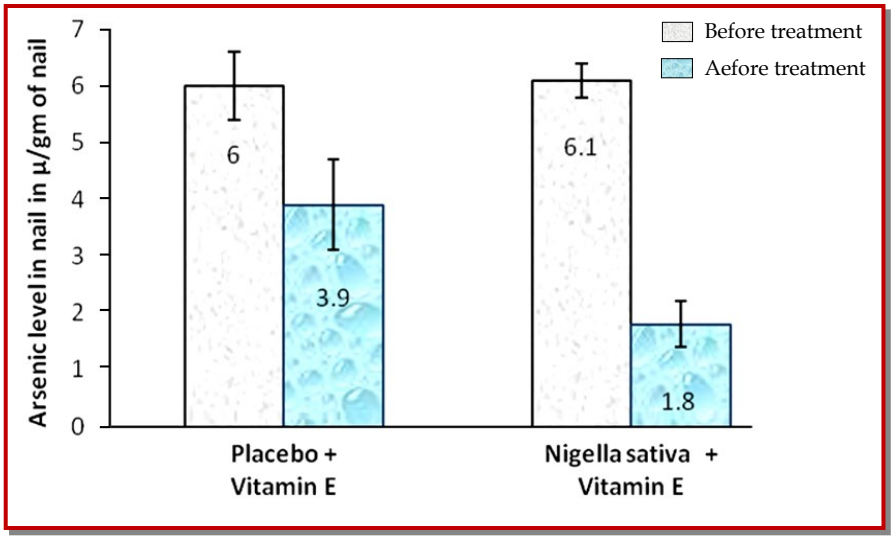

Figure 2: Amounts of total arsenic in nails before and after treatment in Group A and B respectively

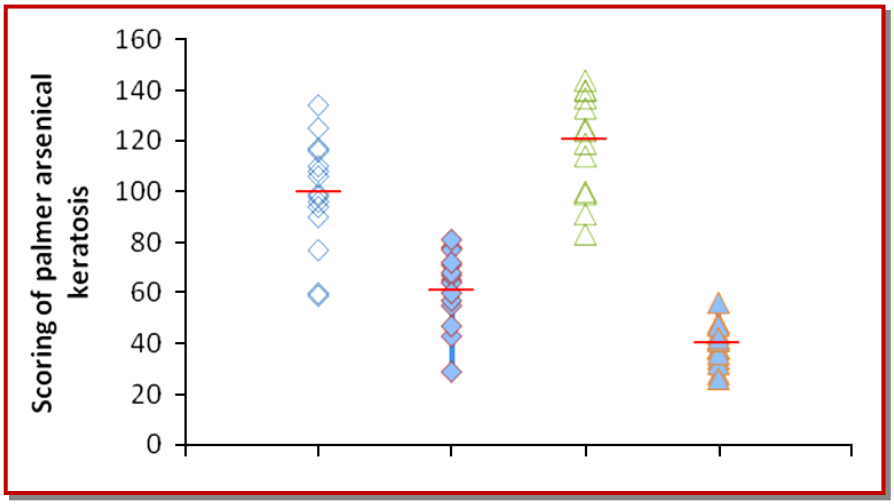

Figure 3: The scoring of palmar arsenical keratosis in Group A and B. Open diamond (before treatment) and close diamond (after treatment) represented Group A. On the other hand, open triangle (before treatment) and close triangle (after treatment) represented Group B. Horizontal red line indicates the mean value

18.4 ± 6.2 years, whereas 20.6 ± 7.3 years in Group B. The mean amount of arsenic in the tube well water of Group A and Group B were 856.9 ± 324.9 and 826.6 ± 231.0 µg/L respectively. The duration of symptoms of palmar keratosis was 12.6 ± 5.2 years in Group A and 13.4 ± 6.8 years in Group B.

The amounts of total arsenic in nail of patients of both groups were almost similar (6.0 ± 0.6 µg/g of nail vs 6.1 ± 0.3 µg/g of nail of Group A and B respectively; Figure 2). Eight week's treatment with only vitamin E reduced the total arsenic level to 3.9 ± 0.8 µg/g of nail (35% reduction) whereas it was reduced to 1.8 ± 0.4 µg/g of nail of Group B (70% reduction).

The mean (± SD) clinical scoring of palmar arsenical keratosis in Group A before and after treatment was 99.3 ± 21.5 and 62.3 ± 14.3 respectively (37% reduction; Figure 3). On the other hand, it was 119.2 ± 20.3 and 39.2 ± 8.4 in Group B (67% reduction). These changes in clinical score in both groups were statistically significant ($p<0.0001$).

After 8 weeks treatment, the reduction of blood glucose level was statistically significant in Group B ($p= 0.0002$) than Group A ($p= 0.1917$). In the patients of Group B plasma lipid profiles (total cholesterol, triglyceride, and low density lipoprotein-cholesterol) were significantly ($p<0.0001$) reduced than before treatment. The percentage of reductions was 23, 23 and 38% respectively. However in Group A, after 8 weeks treatment the reduction was also significant $p= 0.0214$, 0.0019, 0.0051 respectively than before treatment and the percentage of reductions were 8, 13 and 15%. Though after treatment in both group the level of HDL-C (high density lipoprotein-cholesterol) increased significantly ($p<0.0001$), but the percent of increase was

Table II

Metabolic markers in group A and B

Parameters	Group A (Placebo + vitamin E) n = 15		% of (↑) or (↓)	P value	Group B (*N. sativa* oil+ vitamin E) n = 13		% of (↑) or (↓)	P value
Parameters	Before treatment	After treatment			Before treatment	After treatment		
Plasma glucose level (mmol/L)	5.9 ± 1.5	5.2 ± 1.7	11% (↓)	0.1917	8.7 ± 3.6	5.8 ± 1.7	34% (↓)	0.0002
Plasma cholesterol level (mg/dL)	162.5 ± 20.3	149.3 ± 15.6	8% (↓)	0.0214	211.9 ± 16.9	164.1 ± 18.6	23% (↓)	<0.0001
Plasma triglyceride level (mg/dL)	140.4 ± 43.3	122.6 ± 33.6	13% (↓)	0.0019	223.4 ± 76.2	172.9 ± 71.4	23% (↓)	<0.0001
Plasma HDL-C level (mg/dL)	30.4 ± 4.5	36.66 ± 4.21	21% (↑)	<0.0001	30.8 ± 2.7	45.6 ± 4.5	48% (↑)	<0.0001
Plasma LDL-C level (mg/dL)	104.0 ± 24.	88.2 ± 13.8	15% (↓)	0.0051	136.3 ± 17.4	83.9 ± 21.8	38% (↓)	<0.0001

Values were expressed as mean ± SD. Student t test was done between before and after treatment

Table III

Number of subjects complaint for adverse effects

Characteristics	Group A (Placebo + vitamin E)	
Number of participants (n)	15	13
Smell of Nigella sativa	-	10
Gastric irritation	7	8
Diarrhea	4	3
Constipation	4	2

Values were expressed as percentage

48% in Group B and 21% in Group A.

During the study period 10 participants (77%) complaint about the smell of *N. sativa* in Group B (Table III). 47% in Group A and 62% in Group B had gastric irritation (abdominal cramp, indigestion). Only 23% and 15% in Group B and 27% in Group A complaint for diarrhea and constipation.

Discussion

The study result showed that oral administration of *N. sativa* oil capsules twice daily for 8 weeks is affective for the clinical improvement of palmar arsenical keratosis which is reflected by decreasing total arsenic load in nail as well as mean clinical scoring. This is the first report showing that *N. sativa* is affective in arsenical keratosis. The exact mechanism of *N. sativa* against arsenicosis is not known.

Cellular proteins, lipids, carbohydrates even nucleic acids are targeted by free radicals followed by oxidative stress. In 1998, Chen et al., explained that trivalent arsenic toxicity occurred either by attacking –SH groups or by generating reactive oxygen species (ROS). However, since 1990, oxidative stress is represented as a comparatively new theory for arsenic toxicity (Flora et al., 2005; Kitchin, 2001; Flora, 1999). *N. sativa,* containing carbohydrates, proteins, fats, oils has been used for medicinal purpose for centuries. Burits and Bucar (2000) stated that in DPPH assay *N. sativa* possess electron donating capacity and hydroxyl radical scavenging properties. Thymoquinone, one of the active compounds of essential oil of *N. sativa*, not only inhibits non-enzymatic lipid peroxidation (Houghton et al., 1995) but also improves anti-oxidant enzymes status and cellular proteins oxidation process (Ebru et al., 2008). Besides thymoquinone, several compounds like carvacrol, t-anethol, 4-terpineol etc. possessing antioxidant properties (Burits and Bucar, 2000). Its linoleic acid, phenolic compound is also an antioxidant rich compound having lipid peroxidation inhibition capacity. Experimental study revealed that *N. sativa* can remove arsenic (both arsenite and arsenate) ions from waste water (El-Said et al., 2009).

In this study, we also found that *N. sativa* significantly reduces plasma glucose level 34% and plasma lipid profile (total cholesterol 23%, triglyceride 23% and LDL -C 38%) and significantly increases plasma HDL-C 48% in arsenicosis patients. Our findings were in good harmony with those of the studies conducted by Parhizkar et al., 2011; Najmi et al., 2008; Hawsawi et al., 2001. Al-Hader et al. (1993) also approved that volatile oil of *N. sativa* reduced blood sugar in both normal and diabetic rabbit. As *N. sativa* is a source of soluble fiber, which reduced plasma cholesterol either by stimulating bile acid excretion (Bamosa et al., 1997) or inhibiting cholesterol synthesis in hepatocytes (Bamosa et al., 2002) and improved blood glucose and insulin levels (Hawsawi et al., 2001) by improving insulin sensitivity (Meddah et al., 2009; Le et al., 2004).

Conclusion

Depending on clinical improvement of palmar arsenical keratosis and amount of arsenic in nail that is arsenic load in the body, this study shows that *N. sativa* oil is effective in the treatment of palmar arsenical keratosis.

Financial Support

Bangabandhu Sheikh Mujib Medical University, Dhaka

Ethical Issue

The protocol was approved by the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University, Dhaka (BSMMU/2013/1015). Informed written consent of each participant was taken after explaining the purpose, nature, potential risks and benefits of the study in easily understandable local language (Bangla).

Conflict of Interest

Authors declare no conflict of interest

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