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Original Research Article **Pharmacology**

Effect of Chloroform Extract of Nigella Sativa Seed in the Treatment of Palmar Arsenical Keratosis

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Abstract

Background: Arsenical keratosis, a chronic skin disorder resulting from prolonged exposure to inorganic arsenic, poses a significant public health challenge in arsenic-endemic regions like Bangladesh. Characterized by hyperkeratotic lesions, particularly on the palms and soles, the condition is a known precursor to skin cancer. Current treatment options offer limited efficacy, necessitating the exploration of alternative therapies. ***Objective:*** This study aimed to investigate the dose-dependent cytotoxicity of *Nigella sativa* seed extract and assess its therapeutic potential in the management of palmar arsenical keratosis. ***Methods:*** Conducted over 17 months, this observational study involved 32 patients diagnosed with moderate to severe palmar arsenical keratosis from two high-risk unions in Chuadanga, Bangladesh. *Nigella sativa* seed extract ointment was formulated and applied topically. The primary outcome was the reduction in the size of keratotic nodules, measured before and after 12 weeks of treatment using vernier slide calipers. Arsenic levels in patients' water and nail samples were analyzed using a modified silver diethyldithiocarbamate method. Statistical analyses were performed using paired t-tests and Pearson correlation. ***Results:*** Topical application of *Nigella sativa* seed extract resulted in a statistically significant 72.8% reduction in mean lesion size (from 55.4 ± 29.8 mm² to 15.1 ± 10.8 mm²; $p < 0.0001$). No significant correlation was found between lesion size reduction and duration of arsenic exposure (r = -0.28), duration of lesion appearance (r = -0.17), or arsenic concentration in drinking water (r = -0.19). Patient adherence to treatment was high (mean adherence: 94.0 ± 6.6%), and only one case of mild, transient local irritation was reported. ***Conclusion:*** The findings demonstrate that *Nigella sativa* seed extract exhibits dose-dependent cytotoxicity against hyperproliferative keratinocytes, making it a promising, well-tolerated topical therapeutic for palmar arsenical keratosis. Compared to previous treatments, this natural formulation offers superior efficacy and minimal side effects, warranting further investigation through larger clinical trials.

Keywords: Arsenical keratosis, Nigella sativa, Cytotoxicity, Topical therapy.

INTRODUCTION

Arsenical keratosis is a chronic dermatological condition caused by prolonged exposure to inorganic arsenic, typically through contaminated drinking water or occupational hazards. Characterized by hyperkeratotic lesions primarily on the palms and soles, this condition not only disfigures but also serves as a precursor to skin malignancies. In arsenic-endemic regions, especially in parts of South Asia, the incidence of arsenical keratosis continues to pose a major public health concern due to limited treatment options and persistent environmental exposure [1-3].

Current therapies for arsenical keratosis focus largely on symptom management, chelation therapy, and long-term monitoring. However, these interventions often yield suboptimal results, prompting the exploration of alternative and adjunct therapies. In recent years,

natural compounds with antioxidant and anti-inflammatory properties have attracted attention for their potential role in mitigating arsenic-induced damage. Among these, *Nigella sativa*, commonly known as black seed, has emerged as a promising candidate due to its broad pharmacological activities [4-7].

Nigella sativa seeds are rich in bioactive compounds such as thymoquinone, nigellidine, and carvacrol, which have demonstrated notable anti-cancer, anti-inflammatory, and antioxidant properties. These phytochemicals may provide cellular protection against oxidative stress and DNA damage, both of which are hallmarks of arsenic toxicity [7-8]. Importantly, preliminary studies suggest that *Nigella sativa* extract may induce cytotoxic effects selectively in abnormal or transformed cells, raising the possibility of its therapeutic application in precancerous skin conditions like arsenical keratosis [9-10].

The concept of dose-dependent cytotoxicity is critical when evaluating the safety and efficacy of *Nigella sativa* extract. A controlled and gradual increase in dose can help identify a therapeutic window where the extract is potent against keratinocyte proliferation without harming healthy skin cells. Understanding this relationship is crucial for translating laboratory findings into clinically viable treatments, particularly in vulnerable populations exposed to environmental toxins.

OBJECTIVE

This study aims to evaluate the dose-dependent cytotoxic effects of *Nigella sativa* seed extract in vitro and assess its potential therapeutic benefits in individuals affected by arsenical keratosis.

METHODOLOGY

Type of Study

This research was conducted as observational study aimed at evaluating the therapeutic efficacy of *Nigella sativa* seed extract ointment in the management of arsenical keratosis.

Study Location

The field component of the study took place in Chuadanga district, identified by the Public Health Engineering Department of Bangladesh as a high-risk area for arsenic contamination. Specifically, Begampur and Titudah unions under Chuadanga Sadar Upazila were selected due to their high burden of arsenicosis cases. These unions are situated approximately 213 km and 204 km from Dhaka, respectively. Laboratory activities—including water and nail sample analysis for arsenic content, seed extract preparation, and formulation of the ointment—were carried out at the Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbagh, Dhaka.

Study Duration

The study was conducted over 17 months, commencing in September 2019 and concluding in January 2021.

Protocol Development and Ethical Approval

An extensive literature review informed the development of the study protocol. Under faculty supervision, the protocol was refined and submitted on December 18, 2019, to the Institutional Review Board (IRB) of BSMMU. Feedback was received during a subcommittee review on January 4, 2020, and the final protocol was approved on February 3, 2020 (IRB Registration Number: BSMMU/2020/1642).

Informed Consent

All participants diagnosed with arsenical keratosis were thoroughly informed in simple Bengali about the study's objectives, methodology, duration, potential outcomes, benefits, and possible risks. The ointment application process was explained in detail. Written consent was obtained via signature or thumb impression, and participants retained the right to withdraw at any stage. Personal data confidentiality was strictly maintained.

Participant Enrollment

Prior to participant recruitment, the principal investigator completed a one-week observational training at the Dermatology Department of BSMMU, focusing on various dermatoses including arsenical keratosis. A total of 34 patients with arsenical keratosis were enrolled—9 from Titudah and 25 from Begampur—according to predefined inclusion and exclusion criteria (Ferdoush & Misbahuddin, 2014).

Inclusion Criteria:

- Age between 19–65 years
- Either gender
- Clinically confirmed moderate to severe palmar arsenical keratosis
- History of drinking arsenic-contaminated water (>50 μg/L) for more than 6 months
- No topical treatment within the previous 3 months
- Willingness to participate and comply with study protocols

Exclusion Criteria:

- Age below 19 or above 65 years
- Pregnant or lactating women
- Recent use (within 3 months) of systemic or topical treatment for arsenicosis
- Known allergy to *Nigella sativa*
- Other dermatologic conditions such as psoriasis, eczema, or atopic dermatitis
- Diagnosed systemic diseases (e.g., diabetes, lupus, hepatitis, rheumatoid arthritis)

Rationale for Study Site Selection

Chuadanga was selected based on its high prevalence of arsenicosis and cooperation from local health authorities. According to the 2013 report by the Directorate General of Health Services (DGHS), 550 arsenicosis cases were recorded in Chuadanga Sadar Upazila, comprising 302 males and 248 females.

Study Parameters

The primary outcome measure was the size of palmar keratotic nodules, assessed before and after treatment using vernier slide calipers (Tricle, China). Additionally, arsenic concentrations in drinking water and nail samples were analyzed using a modified silver diethyldithiocarbamate method.

Sampling Procedure

Of the 550 recorded arsenicosis cases, 223 individuals exhibited keratotic lesions. Among them, 78 were from the targeted unions (Titudah and Begampur). After screening, 34 participants were enrolled, with 32 completing the study and 2 dropping out.

Study Procedure

Field Work: The investigator collaborated with the Upazila Health and Family Planning Officer and a Health Inspector, who provided a patient registry. Patients were followed at baseline, during, and after the treatment phase.

Data Collection: Data were captured using a structured case report form, documenting demographic details, medical history, and arsenic exposure. Physical examinations and photographic documentation of lesions were also performed.

Water Sample Collection: Participants submitted 100 ml of drinking water in clean, labeled plastic bottles, which were refrigerated until analysis.

Nail Sample Collection: Participants were instructed to grow out nails, wash hands and feet prior to clipping, and submit 200–500 mg of nail samples in labeled polyethylene bags. Samples were stored under refrigeration for later analysis.

Arsenic Estimation Method: The modified silver diethyldithiocarbamate method involved acid digestion of samples, arsine gas generation, and formation of a red-colored complex, which was quantified spectrophotometrically at 535 nm.

Statistical Analysis

Data were presented as mean ± standard deviation (SD). Pre- and post-treatment comparisons of nodule size were analyzed using a paired t-test in Microsoft Excel.

RESULTS

This table provides a summary of the demographic and exposure characteristics of the 32 patients diagnosed with palmar arsenical keratosis. The average age was approximately 43 years, with a male-to-female ratio of 5:3.

Table-1: Characteristics of Palmar Arsenical Keratosis Patients

Parameter	Value
Total number of patients	32
Male patients (n)	20
Female patients (n)	12
Mean age (years ± SD)	43.1 ± 11.5
Mean duration of arsenic exposure (years ± SD)	20.5 ± 7.7

Among 32 patients the mean (± SD) size of keratotic nodules was 55.4 ± 29.8 mm^2 before applying the ointment and the size reduced to15.1 ± 10.8 mm^2 after end of the treatment. The total reduction occurred 72.8% and the p-value was <0.0001 which was statistically significant. The statistical analysis was done by paired t-test.

Table 2: Measurement of the size of keratotic nodular lesions before and after applying the ointment

Before (mm^2)	After (mm^2)	p-value	% net reduction
41.6	26.5		
37.4	17.2		
140.6	34.0		
71.4	15.8		
47.0	0.0		
29.6	16.5		
34.4	12.5		
55.6	20.6		
54.1	20.3		
66.3	15.3		

74.4	19.3	<0.0001	72.8
115.4	39.3		
30.9	0.0		
34.6	9.3		
61.3	23.3		
34.3	0.0		
39.3	16.4		
69.0	16.3		
135.3	29.0		
105.0	29.2		
37.1	0.0		
59.6	0.0		
43.7	12.4		
29.9	0.0		
53.8	21.6		
33.7	13.8		
39.9	15.0		
52.1	17.6		
36.0	19.8		
31.9	0.0		
47.5	21.5		
30.3	0.0		
55.4 ± 29.8	15.1 ± 10.8		

The relationship between duration of arsenic exposure and percentage reduction of lesion size of 32 patients were plotted. The correlation coefficient, r between these two variables was calculated using excel and the value was -0.28 (p- value >0.05). In patients with arsenical keratosis, 20 patients were found to arsenic exposed for about ≤20 years and their reduction of keratotic lesion size was 75.0%, 12 patients were found to arsenic exposed for about >20 years and their reduction of keratotic lesion size was 69.6%.

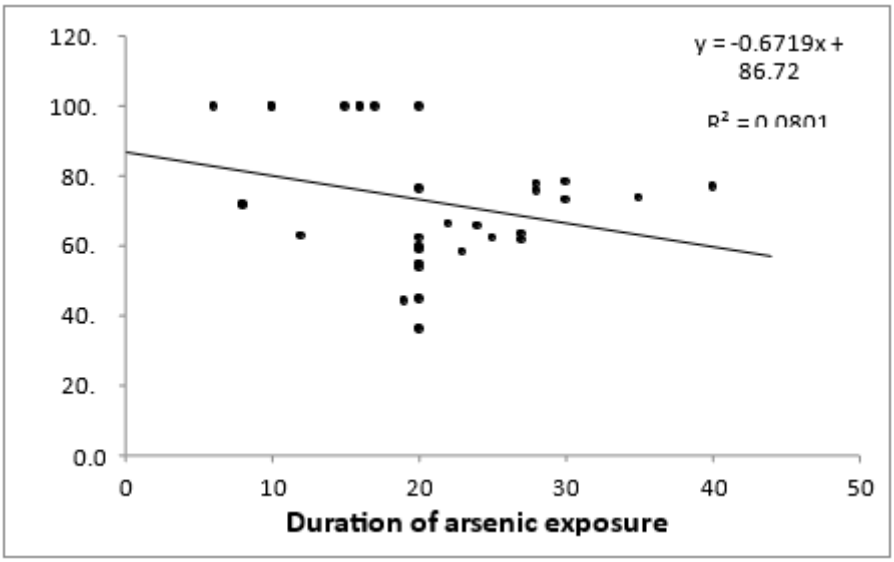

Figure-1: Scatter diagram shows the correlation of percentage reduction of lesion size with duration of arsenic exposure

The relationship between duration of appearance of lesion and percentage reduction of lesion size of 32 patients were plotted. The correlation coefficient, r between these two variables was calculated using excel and the value was -0.17 (p- value >0.05). In patients with arsenical keratosis, 17 patients were found with the appearance of skin lesions for about ≤10 years, and their reduction of keratotic lesion size was 76.8%, 15 patients were found with the appearance of skin lesions >10 years, and their reduction of keratotic lesion size was 68.9%.

Table 3: Relationship between the duration of appearance of the lesions and percentage reduction of the lesion size

Duration of appearance of the lesions (years)	Palmar arsenical keratosis (n = 32)			
	Number of patients	%Reduction	r-value	p-value
≤10	17	76.8		
>10	15	68.9	-0.17	0.34

The relationship between concentration of arsenic in drinking water and the percentage reduction of lesion size of 32 patients were plotted. The correlation coefficient, r between these two variables was calculated using excel and the value was -0.19 (p-value >0.05) [17]. patients with palmar arsenical keratosis had the concentration of arsenic in water ≤300 µg/l and their reduction of lesion size was 75.2%. The concentration of arsenic in water was >300 µg/l in 15 patients and their reduction of lesion size was 70.1%.

Table 4: Relationship between the percentage of reduction of the lesion size and concentration of arsenic in drinking water of patients

Concentration of arsenic in water (µg/ l)	Palmar arsenical keratosis (n = 32)			
	Number of patients	%Reduction	r-value	p-value
≤300	17	75.2		
>300	15	70.1	-0.19	0.27

Compliance of patients was 94%, after the completion of twelve weeks of treatment.

Table 5: Percentage of patients' adherence

Patient ID no.	% Adherence
1	97.6
2	100.0
4	92.3
6	95.2
7	100.0
8	94.0
9	89.3
10	100.0
11	95.8
12	88.7
13	94.6
14	82.1
15	100.0
16	97.0
17	81.5
18	94.0
19	100.0
20	100.0
21	81.0
22	82.1
23	99.4
24	96.4
25	95.2
26	93.5
27	97.6
28	79.2
29	100.0
30	100.0
31	85.7
32	100.0
33	98.2
34	95.8
Mean ± SD	94.0 ± 6.6

DISCUSSION

A study was conducted in 2014 to evaluate the effect of *N. sativa* oil in the treatment of mild to moderate palmar arsenical keratosis (Basar *et al*., 2014) [7]. In that study, patients were treated with capsules of *N. sativa* oil (500 mg) and vitamin E (200 mg). The study was continued for eight weeks. At the end of the treatment, it was found that a 67% reduction occurred. So, oral administration of *N. sativa* oil was found effective in arsenical keratosis. But the study was limited to moderate arsenical keratosis and also produces some adverse effects like abdominal cramps, indigestion, diarrhea, and constipation. In this study *N. sativa* seed extract applied topically is not only moderate but also in severe palmar arsenical keratosis and a 72.8% reduction of the keratotic nodule was observed. Only one patient complained about a mild burning sensation during applying the ointment which spontaneously resolved within a few minutes and the condition occurred for two days. The compliance of the patient was 94% in the present study which was not mentioned in the previous study. In the previous study, thin layer chromatography was not done.

To assess an effective treatment in arsenical keratosis several studies were conducted. Folic acid supplementation causes reduced blood arsenic level where arsenic is methylated to monomethyl arsenic and dimethyl arsenic acids and excreted through urine. 7 (Gamble *et al*., 2007). But depression, skin rashes, nausea, vomiting may develop from folic acid supplementation. In a study, a soft capsule of garlic oil administered orally for twelve weeks was found effective in arsenical palmar keratosis but it produced gastric irritation in half of the participants and also had a bad smell. In cells, arsenic binds with the sulfhydryl group. Garlic oil contains sulfur which binds with the sulfhydryl group and displaces arsenic (Misbauddin *et al*., 2013). 8Topical application of propylene glycol was also found effective on arsenical keratosis but 15% of patients complained about itching. Propylene glycol acts as keratolytic and maintains a normal desquamation process by retaining water from the deeper dermis (Dina and Misbauddin, 2011) [8-9].

Topical application of 20% and 30% salicylic acid found effective in keratosis. Salicylic acid acts as a keratolytic agent but long-term administration with high concentration causes pruritus and burning sensation (Islam *et al*., 2007) [10]. Low dose acitretin and salicylic acid combination orally administered to observe its effect on arsenical keratosis (Son *et al*., 2008) [11]. In that study, severe mucocutaneous reaction was reported. An ointment containing *Azadiractha indica* extract on palmar arsenical keratotic nodular lesion was found effective. It acts by inhibiting the proliferation of keratinocytes. In that study burning sensation and foul-smelling of the extract was the disadvantage (Ferdous and Misbauddin, 2014) [8]. Effectiveness of solasodine of *S. melongena* peel extract (Sultana and Misbauddin, 2020) and compound isolated from cock's comb extract (Sharmin and Misbauddin, 2020) were also studied in arsenical keratosis and found effective with slight burning in skin. *S. melongena* peel extract reduces the keratotic lesion by possessing antioxidant, and anticancer activity where cock comb's extract reduces the lesion size by desquamation process [8].

N. sativa contains protein, oil, fat, carbohydrate which has medical properties and is used as medicine for a long period. There are more than a hundred compounds present in it. Among them, some notable compounds are thymoquinone, dithymoquinone, thymohydroquinone, thymol, alpha-hederin, carvacrol, t-anethole, 4-terpineol, p- cymene, nigellimine-N-oxide, nigellicine, nigellidine etc. Cellular lipids, carbohydrates, proteins, and even nucleic acid are damaged by free radicals following oxidative stress. Arsenic toxicity occurs by generating reactive oxygen species.

Trivalent arsenic (arsenite) toxicity caused by attacking sulfhydryl group, inhibits glutathione reductase and diminished intracellular level of reduced glutathione (Chen *et al*., 1998) [13]. Arsenic causes cell damage by increasing reactive oxygen species and free radicals like hydrogen peroxide, nitric oxide, superoxide anion, hydroxyl radical and produces oxidative stress (Flora *et al*., 2005).

CONCLUSION

The study demonstrated that *Nigella sativa* seed extract ointment significantly reduced the size of palmar arsenical keratotic nodules, with a mean reduction of 72.8% (from 55.4 ± 29.8 mm² to 15.1 ± 10.8 mm²; $p < 0.0001$). While no statistically significant correlations were found between lesion size reduction and the duration of arsenic exposure, duration of lesion appearance, or arsenic concentration in drinking water, patients with shorter exposure and lesion history experienced slightly greater improvements. Additionally, patient compliance was high, with a mean adherence rate of $94.0 \pm 6.6\%$, indicating good acceptability of the ointment. These findings suggest that the *Nigella sativa* ointment is a promising and well-tolerated therapeutic option for managing arsenical keratosis.

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