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Use of topical Purified Solasodine of Solanum Melongena Peel Origin in the Treatment of Moderate to Severe Palmar Arsenic Keratosis

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Key words:
Solasodine, Solanum melongena, brinjal peel origin, palmar arsenic keratosis

Abstract:

Background: *Bangladesh has one of the highest rates of arsenic poisoning in the world. Arsenicosis often manifests as arsenic keratosis on the sole and palm of the hand. Brinjal (solanum melongena) is a common vegetable in Bangladesh and elsewhere. A study demonstrated that an ointment comprising a crude extract of Solanum melongena peel is effective in treating arsenic keratosis. This study was conducted to use the particular component present in brinjal peel which can cure arsenic keratosis.* ***Methods****: The Bangladesh Medical University department of pharmacology reported that S. melongena peel crude extract topical ointment was helpful in arsenical keratosis in two tests. The present study observed arsenic keratosis lesions or nodular size following 12 weeks of pure solasodine ointment therapy. A total of 20 patients were enrolled based on the established inclusion and exclusion criteria. Of the patients, 14 exhibited severe keratosis, while 6 presented moderate keratosis. One patient with arsenic keratosis discontinued participation during the follow-up period due to complaints of heaviness at the application site. Nineteen patients completed a 12-week therapy regimen.* ***Results:*** *This prospective observational clinical trial reports that patients with palmar arsenical keratosis exhibited a mean (± SD) keratotic skin lesion size of 31.7±12.4 mm² prior to the intervention and 7.2±6.8 mm² following the intervention. The reduction percentage was 77.6 ± 19.8, with a p-value of 0.0001. Statistical analysis indicated significance.* ***Conclusion:*** *The research that proved solasodine, a compound found in S. melongena peel, was what heals arsenical keratosis lesions.*

Introduction:

Arsenicosis has been a neglected disease for many years, so satisfactory treatment did not emerge. About millions of Bangladeshi people are exposed to an arsenic concentration above safety level through contaminated drinking water.[1]

Keratosis of the palms and soles is one of the manifestations of chronic arsenic exposure that impairs the functional ability of the patients and affects their socio-economic condition.[2] Until now there has been no satisfactory treatment for keratosis. This study was carried out in search of a better alternative treatment for keratosis.

Solanum melongena (brinjal or eggplant) is a common vegetable found in different parts of the world. *S.melongena* peels contain anthocyanins-delphinidin, nasunin, steroidal alkaloids.[3] Previously, two studies were conducted in the Pharmacology department of Bangladesh Medical University to see the effect of crude extract in the topical treatment of arsenic-induced keratosis.[4] The present study was conducted to see the effect of a purified solasodine compound in the topical treatment of arsenic-induced keratosis. The present study revealed that solasodine isolated from S. melongena peel effectively treated palmar arsenical keratosis.

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Methods:

A prospective cohort Study was in progress from September 2018 up to January 2021. The first four months (from September 2018 to December 2018) were taken to select the thesis title and submission of the protocol to the Institutional Review Board for approval. Six months were required for the extraction procedure and enrollment of patients. One month was needed for the identification of solasodine. Six months were required to prepare and distribute ointment and observation of the response of treatment, writing, and submission of the thesis to the Dean of the Faculty of Basic Science and Paraclinical Science.

This study was carried out at Bangladesh Medical University, and patients selected from Babutipara,Kamalla and Yousufnagar union of Muradnagar Upazilla of Cumilla district. Lab procedures and analysis were done in the Department of Pharmacology, Bangladesh Medical University, Shahbag, Dhaka. Infrared spectroscopy (IR) and Nuclear magnetic resonance (NMR) spectroscopy were done in Wazed Miah Science Research Center, Jahangirnagar University, Savar, Dhaka.

Twenty patients enrolled according to inclusion and exclusion criteria. Inclusion criteria were the presence of moderate to severe palmar arsenical keratosis, drinking arsenic-contaminated water for at least six months and agreed voluntary to participate who are between 18-60 years of age. Patients excluded from the study were pregnant and nursing mother, patient allergic to brinjal.

The arsenic was measured from water and nail samples collected from the patients. Solasodine was extracted (45 grams) after processing peels of 145 kg Solanum melongena. Ethanol extract of S. melongena peel was treated subsequently with concentrated acid and ammonia, then washed with water. The solid part of the extract dissolved in chloroform, and solasodine isolated. Then a cytotoxic study was done with the brine shrimp lethality test. Thin-layer chromatography, infrared spectroscopy, and nuclear magnetic resonance spectroscopy of the extract confirm the presence of solasodine esters (Figure 1&2).

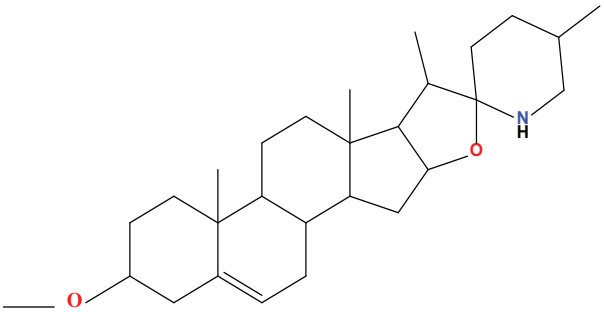

Figure 1 : *Ester derivative of Solasodine*

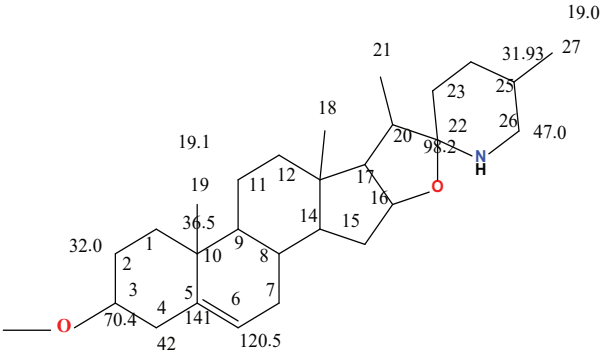

Figure 2: *^{13}C NMR of Ester derivative of solasodine*

The cytotoxic effect of solasodine derived from S. melongena peel extract was evaluated using brine shrimp assays. The brine shrimp lethality test serves as a quick, cost-effective, and practical bioassay for evaluating active plant constituents (Meyer et al., 1982; Sarah et al., 2017).[5,6] The solution with a higher concentration of solasodine extract exhibited a greater number of dead nauplii.

A topical ointment (Figure-3) was prepared with solasodine extract and supplied to the patients. The

Figure 3: Ointment prepared with solasodine extract

patients were demonstrated how to apply the ointment twice daily for 12 weeks. Follow up given regularly through phone calls and adherence and adverse effects noted through the study period. Photographs were taken, and the keratotic nodules' size was measured by slide calipers before and after completion of the treatment.

Operational definition:

Solasodine: A toxic alkaloid, is found in Solanaceae plants including eggplants, potatoes and tomatoes. Research found that it has cytotoxic and anticancer properties. Research into the possible medicinal uses of solasodine is continuing.

Results:

A total of 145 kilograms of brinjal (S. melongena) produced 45 grams of solasodine. Yield 0.03 percent.

Table I : *Physical characteristics of the solasodine extract*

Color	Violet
Consistency	Semisolid
Odor	Pungent
Flavor or taste	Bitter

Brine shrimp experiment:

The solution with a higher concentration of solasodine extract exhibited a greater number of dead nauplii. This suggests that it possesses cytotoxic properties, thereby demonstrating keratolytic effects in humans (Table-II).

Table II: 24-hour brine shrimp lethality test results for various solasodine doses as measured by the number of dead nauplii (Table II)

The concentration of the solasodine extract (µg/ mL)	Percentage of dead nauplii at the end of 24 hours
0.0	10%
100.0	100%
10.0	80%
1.0	60%
0.1	50%

A single spot was observed in the TLC plate by thin layer chromatography (Figure-4), which confirms the presence of a pure solasodine extract.[7]

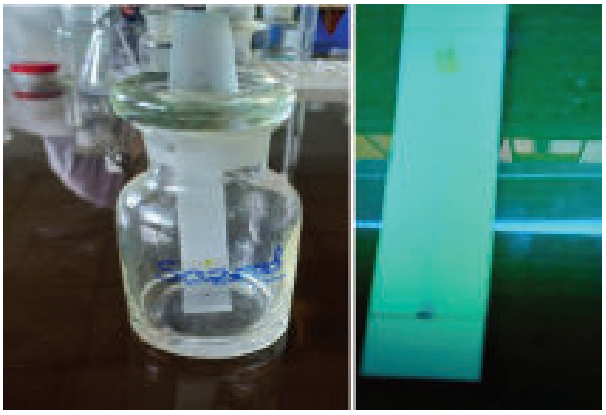

Figure 4: A) Thin layer chromatography of solasodine extract. B) TLC plate under UV light showing a single spot

In total, 20 patients enrolled according to the inclusion and exclusion criteria. Among them, 14 patients were with severe keratosis, and 6 patients were with moderate keratosis. One patient with arsenic keratosis dropped out during the follow-up period complaining about heaviness at the application site. A total of 19 patients completed 12 weeks of therapy.

The mean (± SD) age of the patients was 38.5 ± 11 years. The mean arsenic amount in tube well water was 244.1 ± 177.5 µg/L, and the mean arsenic amount in nail was 25.2 ± 3.8µg/gram. The mean duration of exposure to arsenic-contaminated water of the patients was 19.4 ± 6.0 years. The mean duration of the appearance of keratosis was 7.6 ± 3 years.

Table III: Characteristics of the patient parameters of arsenic keratosis

Parameters	Palmar arsenical keratosis (n=19)
Male patients (n)	8
Female patients (n)	11
Age (years)	38.5 ± 11.0
Amount of arsenic in tube well water (µg/L)	270.8 ± 172.0
Amount of arsenic in the nail (µg/g)	25.2 ± 3.8
Duration of arsenic exposure (yrs)	19.4 ± 6.0
Duration of the appearance of keratosis (years)	7.6 ± 3.0

Data presented as mean ± SD

In patients with palmar arsenical keratosis, the mean (± SD) size of the keratotic nodules was 31.7±12.4 mm^2 before the intervention, and the mean size was reduced to 7.2±6.8 mm^2 after the intervention. The percentage of reduction was 77.6±19.8. Statistical analysis was done by paired t-test, and the p-value was 0.0001. The result was statistically significant (Table IV).

Table IV : Measurement of the size of the keratotic nodules

Size of the keratotic nodules in patients with palmar arsenical keratosis (n=19)			P-value
Before intervention (mm^2)	After intervention (mm^2)	% Reduction	
68.30	16.1	76.4	
27.65	12.8	53.7	
32.78	7.5	77.1	
41.78	8.3	74.8	
13.53	0.0	100.0	
24.60	6.2	74.7	
21.50	0.0	100.0	
32.22	0.0	100.0	
38.51	20.8	46.0	
38.25	5.3	86.1	< 0.0001
32.32	0.0	100.0	
48.64	21.3	56.0	
28.50	6.7	76.3	
35.25	0.0	100.0	
30.58	4.7	84.6	
21.73	7.8	64.1	
27.80	8.6	69.0	
17.95	11.3	37.0	
19.96	0.0	100.0	
31.7 ± 12.4	7.2 ± 6.8	77.6 ± 19.8	

Table V : Distribution of patients according to the duration of arsenic exposure

Duration of arsenic exposure (years)	Number of patients (n=19)	%Reduction of the size of the lesion	r-value	p-value
01-20	11	74.9	+0.105	0.668
>20	8	80.3		

This result indicates that the ointment containing solasodine extract is effective in both short and long arsenic exposure.

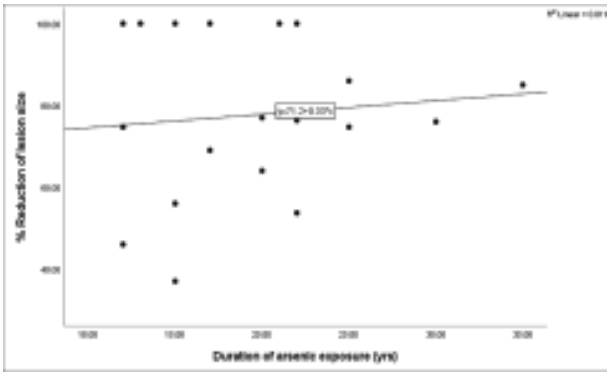

Figure-5: Scatter diagram showing the correlation of percentage reduction of the lesion with the duration of arsenic exposure (years)

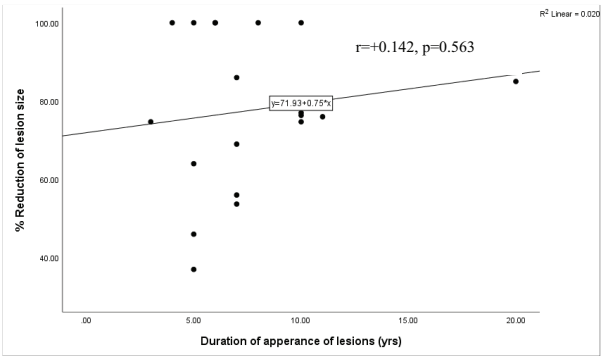

Figure-6: Scatter diagram showing the correlation of percentage reduction of the lesion with the duration of appearance of lesions (years)

Table VI : Distribution of patients according to the duration of appearance of the lesions and reduction of the lesion size

Duration of the lesions in years	Number of patients (n=19)	%Reduction	r-value	p-value
1-10	17	75.3%	+0.142	0.563
>10	2	80.4%		

Size before treatment = 28.5 mm2, size

After treatment = 6.7mm2

Figure-7

Size before treatment = 30.6 mm2

Size after treatment = 4.7mm2

Figure 8

Figure 7&8: Sample photograph of patients; showing the condition prior to and following the application of the ointment formulated with purified solasodine extract.

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Discussion:

In this study, patients with palmar arsenical keratosis had a mean (± SD) keratotic skin lesion size of 31.7±12.4 mm^2 before and 7.2±6.8 mm^2 after the intervention. The reduction percentage was 77.6±19.8, with a p-value of 0.0001. Three studies have examined S. melongena peel extract's effects on arsenical keratosis. The initial trial at the same department demonstrated 53% keratosis improvement with whole extract ointment (Sarah, 2018). It improved 69.8% in the second research using S. melongena peel crude extract ointment. [8] Those investigations showed no significant adverse effects, although 10% of patients in the second study reported slight burning at the application site, which was manageable. Solasodine was found in the crude extract, and it was suggested that it could treat arsenic-induced keratosis.[8]

In this trial, solasodine-containing ointment improved 77.6% arsenic keratotic palm skin lesions. The use of purified solasodine in the ointment instead of crude extract made the improvement more than in those two tests. The ointment was well tolerated, with only one patient reporting palm heaviness.

Among five patients with moderate keratosis, the reduction percentage of the lesion size was 75%, and among fourteen patients with severe keratosis, it was 80.2%. Inference cannot be made with only five patients with moderate keratosis that the ointment is less effective in moderate keratosis. But, this inference can be made that solasodine ointment is significantly effective against severe keratosis.

The study could not achieve any significant relationship between the concentration of arsenic in sample water and lesion size reduction. There was also no meaningful relationship found between the duration of arsenic exposure and the reduction of lesion size. The present study tried to detect the relationship between the period of the appearance of keratosis and the reduction of lesion size. But no conclusion could be reached.

The cytotoxic effect of solasodine of *S. melongena* peel extract was assayed in the brine shrimp. Brine shrimp lethality test is a rapid, inexpensive, and convenient bioassay for active plant constituents. [9,10] There was an increased number of dead nauplii in the solution of the higher concentration of solasodine extract.

The *S. melongena* peel extract's solasodine presence was based on data obtained from nuclear magnetic resonance spectroscopy and infrared spectroscopy. An ester derivative of solasodine was identified from the data. In NMR spectroscopy, chemical shifts present correspond to the structure of the ester derivative of solasodine. Chemical shifts are quite similar and within the reference value of the studies done previously. Esterification occurs due to the treatment of *S. melongena* peel extract with concentrated acid during Soxhlet extraction.

As solasodine glycoside kills cancer cells, a mixture of solasodine glycoside was also used at low concentration (0.005%) to treat solar keratosis, squamous cell carcinoma, and basal cell carcinoma. At the end of 13 weeks of treatment, all the lesions were cured clinically and histologically.[11]

Solasodine, solamargine, and solasonine are steroidal alkaloids in S. melongena peels.[12] These substances moderately to strongly inhibited carbon tetrachloride-induced hepatocellular cancer in rats. Solasodine glycoside may destroy cancer cells by lysis after expressing endogenous endocytic lectins. The component is more selective to cancer cells than normal cells and induces apoptosis by upregulating tumor necrosis factor and Fas receptor .[11]

Anthocyanin, delphinidin-3-(p-cumaroylrutinoside)-5-glucoside, and nasunin in S. melongena peel are antioxidants.[13] Delphinidine inhibits HT-1080 fibrosarcoma invasiveness in vitro .[14]

The effectiveness of garlic oil in the treatment of mild and moderate palmar arsenical keratosis was assessed .[15] This study revealed the reduction of the keratotic nodules' size and the reduction of the total arsenic in nails. However, patients complained about gastric irritation during the whole treatment period.

Another study was conducted to evaluate *Nigella sativa* in patients with moderate palmar arsenical keratosis .[16] The reduction of keratosis was satisfactory, but patients complained about the smell, gastric irritation, diarrhea, and constipation during the study period.

For mild, moderate, and severe palmar arsenical keratosis, a 2007 study examined the efficacy of topical applications of 5%,10%,20%, and 30%

salicylic acid .[17] The keratolytic action of salicylic acid is well-documented. Although the burning sensation was an unpleasant effect, severe keratosis improved more after six months of 30% salicylic acid treatment.

The efficacy of topical 15%, 30%, and 45% propylene glycol for palmar arsenical keratosis was examined in another study .[18]

In 2014, Ferdoush and Misbahuddin examined the effects of topical Azadirachta indica (neem) ethanol extract on palmar arsenical keratosis. A. indica slows keratinocyte growth. A. indica treated with salicylic acid had smaller keratotic nodules.[19] Patients with moderate palmar arsenical keratosis were the subjects of another study that examined Nigella sativa. Although the keratosis reduction was good, individuals had gastrointestinal irritation, constipation, diarrhea, and unpleasant odors throughout the research period.[20] Pure solasodin extract, however, had a much better cure rate.

In the present study, reducing the keratotic nodules' size was achieved by applying ointment containing solasodine extract at the end of 12 weeks without any significant adverse effect. The improvement was assessed clinically, and no follow-up was given after completing the treatment.

In a study, isolated solasodine from Solanum xanthocarpum, showed max(3400, 2950,2880,1733,1693 cm-1) in Fourier transform infrared spectra.[21]

In the present investigation, S. melongena extract Fourier transform infrared spectra (cm-1) were 2924.0 for amine salt, 2852.0 for alkane, 1737 for ester, and 1685 for carboxylic acid.

Similar ^{1}H NMR spectra for solasodine were found in Shabana et al. (2013). [12] Studies found S. melongena fruit peels anti-cancer (Shabana et al., 2013).12 In that investigation, solasodine's ^{13}C NMR spectra were 37.4, 32.0, 71.0, 42.5, 141.5, 120.7, 32.0, 31.5, 50.4, 36.7, 20.9, 39.9, 40.4, 56.5, 31.5, 78.7, 63.2, 15.9, 18.9, 41.4, 14.7, 97.9, 34.2, 30.4, 31.0, 47.5, and 18.9 ppm for C-1 In the current investigation, S. melongena peel extract 13C NMR spectra were 37.1, 32.0, 70.4, 42.0, 141.0, 120.5, 32.0, 31.9, 50.1, 36.5, 21.0, 39.4, 40.4, 56.4, 31.9, 79.0, 61.5, 16.1, 19.1, 41, 15.1, 98.2, 34.4, 30.9, 31.9, 47.0, and 19.2 for C-1 to C- 27. The solasodine spectrum's proton and carbon NMR values are within the reference value. Solasodine esters were found using Chem Draw and NMR data (Figure 3,4).

The study result confirmed that ointment containing purified solasodine extract was more effective than *S. melongena* peel's crude extract in treating moderate to severe palmar arsenical keratosis. The solasodine was the active compound of *S. melongena* peel responsible for the significant improvement of the palm's keratotic skin lesions.

Conclusion:

Compared to the crude extract of S. melongena peel, purified solasodine is more effective in treating moderate to severe palmar arsenical keratosis. Solasodine is responsible for the amelioration of keratotic skin lesions on the palm. So, a potentially economically viable ointment to treat neglected illnesses like arsenical keratosis can be produced if solasodine can be chemically synthesized in the lab.

Limitations:

Due to the Covid 19 pandemic situation, a large sample size and control group cannot be realized at that time. A long-term follow-up of the keratosis patients is necessary to observe the recurrence and confirm the cure rate.

Ethical clearance:

The Institutional Review Board approval registration number: BSMMU/ 2019/ 10655. ClinicalTrials.gov ID NCT04693000

Conflict of interest:

There was no conflict of interest.

Funding:

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