

Investigation of Morphine in Locally Available Sweet Supari Marketed in Karachi, Pakistan

Midhat Rehmani* and Rashid Raza

Department of Food Science and Technology, Jinnah University for Women, Karachi, Pakistan, Postal code-74600

Abstract

The frequency of use of betel nut has been increasing in the city of Karachi, Pakistan for decades and that use is more frequently associated with the chewing of tobacco. New generation has become addicted of using it creating doubts of contamination of addictive in the sweet supari sold in the city. In the present study different brands of sweet supari (chewing areca nut products) which were expected to contain addictive substances were analyzed to determine the levels of morphine. The samples of four different brands of sweet supari were randomly collected from different shops of Karachi, Pakistan. A total of 100 samples i.e. 30 samples of brand 1, 20 samples of each brand 2, brand 3 and brand 4 were taken for detection of opium. Samples were extracted by using standard method of extraction using methanol as an extracting solvent. The analysis of each sample for detection of opium was carried out by using standard methods of analysis by HPLC. The limit of detections (LODs, $n=10$) for the HPLC method were found to be 1.4ng/mL. It is glad to report that the level of morphine in all 100 samples of four various brands was detected less than the detection limit of HPLC. The results of the current study were very encouraging and inspiring that the sweet supari samples are not harmful and dangerous in terms of contamination of addictive. However, other adverse health effects of this silent killer should be taken into account on utilizing it.

Keywords: Areca Catatue, High Performance Liquid Chromatography (HPLC), addiction, morphine

Introduction

East Africa, Asia and the tropical Pacific are famous areas of the world for cultivation of areca or betel nut. It is available in the form of sweet supari and pan (nut wrapped inside betel leaves) in Pakistan. In different area of the country, the composition of pan and supari varies (Humans 2004, Gupta and Warnakulasuriya 2002). The frequency of consumption of this addictive substance areca nut is as much as caffeine, nicotine and wine are consumed. It is reported to be consumed mostly by young and relatively poor children (Boucher and Mannan 2002, Garg, Chaturvedi *et al.* 2014). It is believed to be helpful in digestion by the users, however is not reported in the literature (Boucher and Mannan 2002). Oral health having a great influence by habitual areca nut chewing itself a silent killer and their product chewing can cause deleterious effects on dental and oral health. Tooth fracture periodontal disease, lichenoid lesions leukoplakia, oral sub-mucous fibrosis (Shirzaei and Neshat 2020). In a study it was found that about 74% of primary school children daily chewed areca catechu, indicating that children are the targeted audience of this harmful product. Since there are no regulations addressing the control of areca catechu only the pressure of society can limit the use. In Karachi among poor primary school children the practice of chewing areca catechu is common due to its cheap price (Shah, Merchant *et al.* 2002).

Researches mainly focused on morphine alkaloid present in poppy seeds. As it is biologically most active in the terms of pain relieving but addictive. Among these

alkaloids morphine and codeine are pharmacologically active. The European Food Safety Authority allowed only 10µg/kg body weight of morphine in food products (Carlin, Dean *et al.* 2020). Morphine directly acting on immune system by regulating immune function also play a role for producing of immune mediators. It is the key substance that has a dual role in the regulation of tumors, including it has adverse effect on tumor growth, inflammation, angiogenesis and metastasis (Tuerxun and Cui 2019). Food cravings may be lead to the food addiction which may be due to the certain compound present in the food like specific alkaloids or specifically morphine or codeine. Many of the researches claim that the food addiction is may be due to the presence of addictive components, however no researcher was reported e to prove it (Kuntz, Golombek *et al.* 2021).

Keeping in view the frequency of use and the expected presence of addictive substance in the areca nut; the current study was aimed to analyze expected contamination of addictive substances such as morphine in sweet supari (chewing areca nut products) samples marketed in the city of Karachi, Pakistan, using standard method of analysis. The study would help in formulating regulation for the sweet supari and pan.

Material and Method

Chemicals and Reagents

All chemicals and reagents used in the current study were HPLC grade.

Sample Collection

A total of 100 samples of supari (sweet areca nut) i.e. 30 samples of brand 1, 20 samples of each brand 2, brand 3 and brand 4 were purchased from different local of markets located in the city of Karachi, Pakistan.

Sample Preparation

10g of grinded/powdered supari (sweet areca nut) mixed with extraction solvent (methanol with 0.1% acetic acid) was placed into a 100 mL glass flask and sealed with screw cap. The flask was agitated for 60 min in an automatic shaker. The supernatant liquid was extracted through the disposable syringe. Two hundred microliters of the extract were diluted with 700 µL of 2:3 vol/vol mixture of methanol and water and then it was filtered through PVDF membrane having pore size 0.45 µm.

HPLC Analysis

Reverse-phase HPLC (model LC-10ADvp solvent delivery system; auto injection, Shimadzu, Japan) C₁₈ Brownlee reverse phase column (220x4.6mm, particle size 5µm) with C₁₈ guard column (Perkin Elmer) was used with fluorescence detection set at 284nm. The mobile phase was water:acetonitrile:methanol (66:17:17, v/v/v). The oven temperature was maintained to 40°C with a flow rate of 1mL/min and injection volume for standard and sample extracts was kept 30µL. The calibration solution of morphine ranging from 0.04-10 ngmL⁻¹ were prepared in 1 mL 2:3 vol/vol mixture of methanol and water and then it was filtered through PVDF membrane having pore size 0.45 µm. The limit of detection (LOD) of Aflatoxin M₁ for the human breast milk samples was obtained as 200pg/mL⁻¹.

Statistical Analysis

Standard deviation was estimated by using one-way analysis of variance (ANOVA) according to AOAC guidelines. Calibration curves and linear regression curve showed r^2 values above 0.97 indicating good linearity (Williams 1984).

Results and Discussion

Operational conditions are summarized in Table-1. A linear dynamic ranges from, limit of quantification and detection was 4-240ng/ml, 4ng/ml and 1.4 ng/ml respectively, whereas standard deviation was found to be 6.9%.

Table 1: Limits of Detection of HPLC Method

S. No.	Parameters	Values
1.	Linear dynamic range (ng/mL)	4-240
2.	Limit of quantification (ng/mL)	4
3.	Limit of detection (ng/mL)	1.4
4.	Precision (SD %)	6.9

Retention Time

The estimated retention time for morphine by HPLC method was found to be 8.05 minutes (Figure-1).

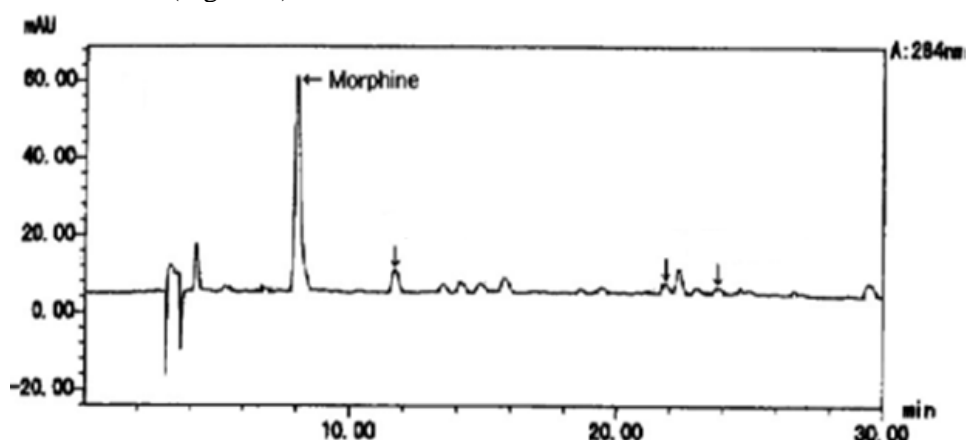


Figure 1: HPLC Chromatogram

Calibration Curve

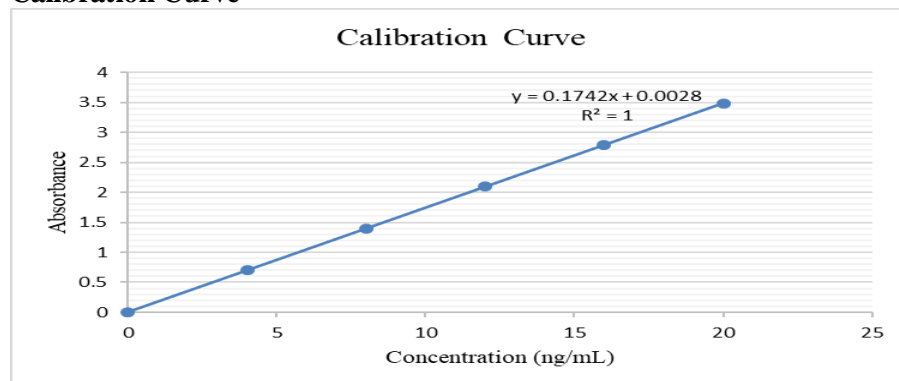


Figure 2: Calibration Curve of Morphine

Analytical Results

The analytical results are summarized in Table-2. A total of 30 samples of brand 1, 20 samples of each brand 2, brand 3 and brand 4 were taken for detection of morphine. No sample was found to be contaminated with morphine indicating no morphine is added to the supari samples analyzed. The results of the current studies were in accordance with the expectation (Kuntz, Golombek *et al.* 2021).

Table 2: Analytical Results of Morphine in Supari Samples by HPLC Method

Type of Supari	Samples Analyzed	Samples Contaminated	Positive %	Mean \pm SD (ng/kg)	Concentration range (ng/kg)
Sweet Supari 1	30	0	0	<LOD	<LOD
Sweet Supari 2	20	0	0	<LOD	<LOD
Sweet Supari 3	20	0	0	<LOD	<LOD
Sweet Supari 4	20	0	0	<LOD	<LOD

Conclusion

In the present study different brands of sweet supari (chewing areca nut products) which were expected to contain addictive substances were analyzed to determine the levels of morphine. The samples of four different brands of sweet supari (100) were randomly collected from different shops of Karachi, Pakistan. A total of 100 samples i.e. 30 samples of brand 1, 20 samples of each brand 2, brand 3 and brand 4 were taken for detection of opium. Samples were extracted by using standard method of extraction using methanol as an extracting solvent. The analysis of each sample for detection of opium was carried out by using standard methods of analysis by HPLC. The limit of detections (LODs, n=10) for the HPLC method were found to be 1.4ng/mL and UV-Visible method were 0.2µg/mL. It is glad to report that the level of opium in all 100 samples of four various brands was detected less than the detection limit of HPLC and UV-Visible spectrophotometry. The results of the current study were very encouraging and inspiring that the sweet supari samples are not harmful and dangerous in terms of contamination of addictive, and therefore, the names of brand are not mentioned. However, other adverse health effects of areca nut should be taken in account on utilizing it.

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