Occurrence of Opium Alkaloids in Commercial Herbal Stomach Remedy Preparation

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Abstract

Nine different brands of commercial herbal stomach remedies were screened for the presence of morphine. Detection of morphine was done by thin layer chromatography followed by gas chromatography. It was found that one of the nine brands screened contained morphine. The amount of morphine present in this particular brand was estimated to be 1.2 mg.

Introduction

STOMACHACHE PREPARATION OF various types are used very widely in South East Asia, in particular by the people of Chinese origin. In Malaysia these stomach preparations can be obtained freely from Chinese druggist and sundry shops.

The purpose of the present investigation is to discover if any of these commercially available preparations contain morphine.

Materials and Methods

Nine different brands of stomachache preparations were obtained from the Chinese druggist shop. All nine preparations had been claimed to be effective in relieving stomachache and vomiting, while a few preparations claimed to be additionally useful in relieving cough, dysentry, indigestion and seasickness. The nine brands are assigned names ranging from brand A to brand I and the weight of each single dose is as shown in table 1.

Extraction – Single doses of the individual preparations were each digested with 25 ml dilute hydrochloric acid (1N) at 60° – 80°C for three hours. The aqueous suspension was filtered and the filtrate treated with solid sodium carbonate until the final solution acquired a pH of 8.6. The alkaline solution was then extracted with a chloroform: isopropyl alcohol (IPA) mixture (3:1) twice (1 × 50 ml, 1 × 25 ml). The combined organic phases was concentrated to 1.5 – 2.0 ml under reduced pressure after drying (anhydrous Na_2SO_4). This fraction was expected to contain any phenolic alkaloids that might be present in the original dose.

Table 1
Sample Identification and Their Corresponding Weights

Brand	Weights (g)
A	1.36
B	2.15
C	1.82
D	1.29
E	1.24
F	1.13
G	1.19
H	1.28
I	1.34

Table 2
Morphine Rf Value on Silica Gel TLC (0.1 mm Plates)

Solvent System	Rf Value
$CHCl_3$: IPA, 3: 1, with 1% conc. ammonia	0.45
Benzene: methanol, 4: 1	0.31

Identification – Morphine was identified by means of thin layer chromatography (TLC) on silica gel GF_{254} using the solvent systems indicated. Morphine spots were visualised with iodine vapour (brown spots) and Dragendorf's reagent (orange spots). Only the extract from brand E showed an equivalent spot with identical characteristics (figure 1).

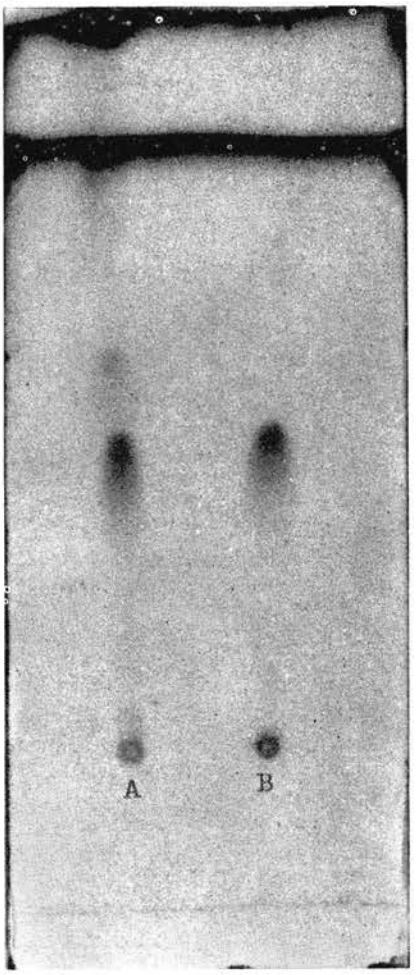

Figure 1
TLC Chromatogram of Sample E (A) and Morphine Standard (B).

Morphine was further confirmed in sample E by conversion of the alkaloid present to its diacetyl derivative (heroin) which was identified separately by comparison on gas chromatography with an authentic sample prepared from morphine.

Quantitative estimation – A quantitative estimation of the amount of morphine present in a single dose of brand E was carried out by measuring the spot

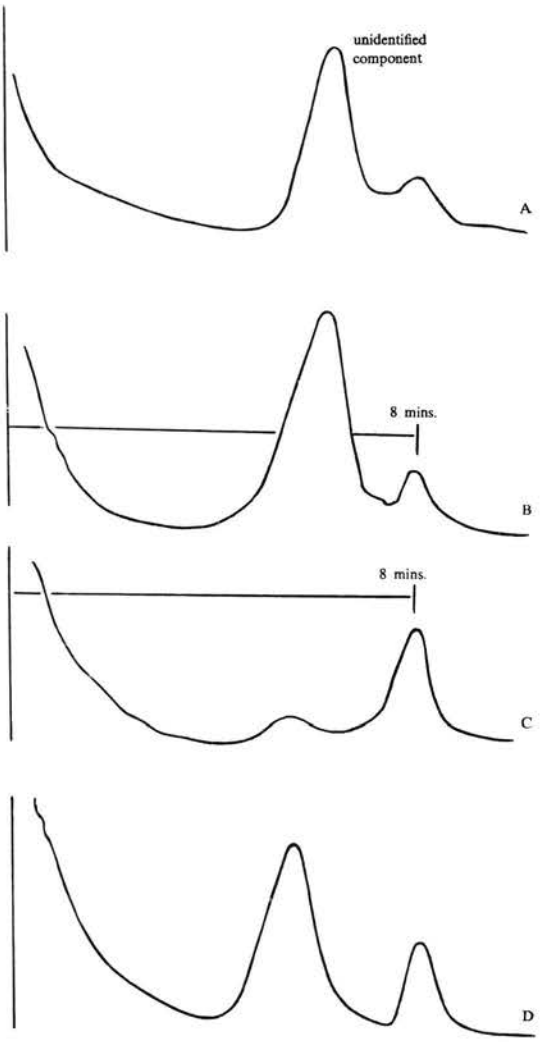

Figure 2
Gas Chromatograms Indicating Presence of Diacetylmorphine.

A = Sample before acetylation
B = Sample after acetylation
C = Diacetylmorphine (heroin)
D = Peak enhancement

areas for known aliquots of a standard solution of E in chloroform. The areas were then compared on a calibration curve obtained with an authentic sample of morphine, figure 3.

Acetylation of morphine standard and of the alkaloid extract from brand E – Morphine sulphate (8.2 mg)

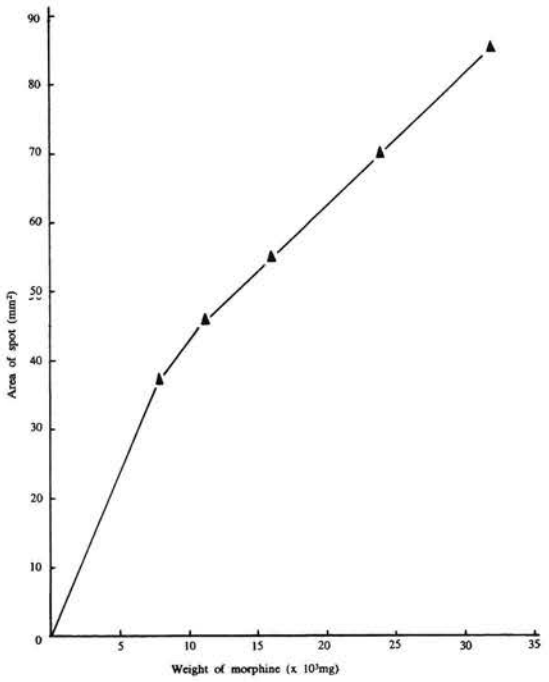

Figure 3
Calibration Curve of Spot Weight VS Area for Morphine on 0.1 mm thick Silical Gel TLC Plates. Spots were visualised with Dragendorf's Reagent.

was dissolved in 0.4 ml acetic anhydride and the mixture refluxed at 155° – 160°C for 5 hours. Excess acetic anhydride was removed under reduced pressure and the residue then taken up in 0.25 ml chloroform containing a trace of ammonia as described by Miller, 1972.

The original extract of brand E in chloroform (1.6 ml) was completely evaporated under reduced pressure. 0.4 ml acetic anhydride was added to the residue and refluxed at 155° – 160°C for 5 hours. Removal of the excess acetic anhydride as before and dissolution of the residue with 0.25 ml chloroform gave the product sample used directly for the gas chromatography.

Gas chromatographic analysis – This was carried out on a 5 feet × $\frac{1}{8}$″, 1.5% OV — 101 on chromosorb Y column at 225°C with nitrogen carrier gas flowing at 26 ml/min using a Varian 940 instrument equipped with a flame ionisation detector.

Results

Of the nine brands of preparation, only the extract of brand E showed an equivalent spot with identical characteristics as shown in figure 1.

In figure 2, trace A is the gas chromatogram of the sample E which does not show the morphine which was expected to be tenaciously retained by the column used. Trace B was obtained from sample E after acetylation; trace C was obtained from the products of the acetylation of morphine and finally trace D in a peak enhancement run with a combined aliquot of acetylated E and heroin. The enhancement of the new peak appearing in trace B confirms that it is heroin and therefore morphine was present in brand E. The TLC supports the conclusion reached with the gas chromatography results in that the morphine spots (Rf 0.20) ($CHCl_3$: IPA, 3:1) were replaced by diacetylmorphine (Rf 0.38).

From the calibration curve of figure 3, an estimate of 1.2 mg of morphine was obtained for a single dose of sample E which corresponded to a content of 0.1%.

Discussions

Both the TLC and gas chromatographic analyses confirmed the presence of opium alkaloids in brand E of the stomachache preparation. An estimate of 1.2 mg of morphine was obtained from the calibration curve with an authentic sample of morphine. It is likely that the actual content of morphine is higher than this as the best recovery of phenolic alkaloids by the procedure described had been shown to be no better than 80% and frequently much lower (Miller, 1972). The limit of detection of morphine by the present method is estimated to be 0.4 mg. The other 8 brands do not contain any morphine or if morphine is present in any, the concentrations do not exceed 0.4 mg/dose.

Conclusions

Nine commercial brands of herbal stomachache preparations were screened for the presence of morphine alkaloids by thin layer chromatography and by gas chromatographic analyses. One brand was found to contain morphine and the concentration present was estimated to be 1.2 mg/dose.

Acknowledgements

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Reference

Miller; R.J., 1972. Ph. D. thesis, University of California, Berkeley, U.S.A.