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Short Communication

The occurrence of acetaminophen/codeine as an adulterant in herbal analgesic supplements in Hamadan, Iran: A pilot study

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ABSTRACT

Analgesics, such as acetaminophen (APAP) and codeine (COD), are used to adulterate medicinal herbs and/or herbal supplements. This study evaluated the APAP and COD levels in 60 herbal supplement and/or herb-based medicine samples collected from apothecaries in Hamadan, Iran. The samples were analysed using a high-performance liquid chromatography (HPLC) system. The results showed that 15% of the samples contained 38900–165200 ng/g and 31.1–603.3 ng/g of APAP and COD, respectively. Due to the side-effects of analgesic drugs in human, control of these drugs is recommended in herbal supplements.

1. Introduction

Medicinal herbs and/or herbal supplements are extensively used to maintain or improve health, and the public believes that natural herbal compounds are safe and have no side effects.[1] The World Health Organization (WHO) reported that approximately 80% of the population in developing countries depend on medicinal herbs for their health needs.[2] However, the illegal adulteration of herbal supplements with synthetic drugs has been frequently reported. For instance, adulterations to herbal supplements, such as analgesics,[3] typically involve the addition of synthetic drugs to make them show faster, more significant pharmaceutical effects, resulting in an increase in product sales.

Pain is an unpleasant sensory and emotional experience related to actual or potential tissue injuries.[4] Many patients take herbal medicines as an alternative or supplementary therapy. However, herbal supplements and/or medicines adulterated with opioid or non-opioid analgesics may have side effects.[3] Acetaminophen (APAP), alone or in combination with codeine (COD), is available as a prescription and over-the-counter drug.[5] However, this synthetic drug may also be illegally added to herbal supplements.[3] Therefore, quantitative APAP/COD monitoring in various medicinal herbs is essential to ensure good health. This study monitored herbal supplements potentially adulterated with APAP/COD in Hamadan, Iran.

2. Materials and methods

2.1. Sampling

A total of 60 samples of medicinal herbs and herbal supplements available in Hamadan, Iran, were collected from local apothecaries during the period of September to November 2017. All samples were kept under suitable conditions and analyzed as soon as possible.

2.2. Apparatus and reagents

APAP and COD in the herbal samples were quantitatively analyzed with a high-performance liquid chromatography (HPLC) system (LC20AD XR, Shimadzu company) equipped with a photodiode array detector (PDA). All solvents and chemicals with a purity of 99% (HPLC grade) were obtained from Merck, Darmstadt, Germany unless otherwise stated. COD (99% purity, CAS#: 76-57-3) and APAP (99% purity, CAS#: 103-90-2) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

2.3. Sample preparation

Two grams of the samples were transferred into a test tube, and

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Table 1
Linearity range, detection limits and summary of method validation data for HPLC analyses.

Drug	Range (ng/ml)	Linear equation	r^2	LOD (ng/ml)	LOQ (ng/ml)	Recovery (%)[a]	RSD (%)[b]
APAP	1000-50000	Y = 186.33x + 220474	0.999	2518	7631	96.47	1.1
COD	1-500	Y = 12234x + 120859	0.999	6.07	18.40	96.11	0.8

LOD: limits of detection and LOQ: limit of quantitation. APAP: acetaminophen, COD: codeine.
[a] The recovery experiments were performed by spiking 100 ng/ml of COD and 100,000 ng/ml of APAP into herbal samples.
[b] Relative standard deviation (RSD) was obtained from five different tests for each experiment.

Table 2
Daily intake values for acetaminophen (APAP) and codeine (COD) in medicinal herbs and herbal supplements.

Drug	Drug concentration (ng/g)			Daily intake (ng/kg body weight)		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
APAP	94,500 ± 55800	38,900	165,200	18900 ± 11170	7780	33040
COD	366.7 ± 258.6	31.14	603.31	73.3 ± 51.7	6.22	120.6

Data was expressed as mean and standard deviation (SD).
Based on the reports collected from Hamadan's apothecaries, the recommended dosage of herbal medicines by druggists is estimated at 12 g/day.

15 ml of methanol was added. The samples were then sonicated at 50 °C for 15 min. Next, the samples were placed in the dark for 48 h. After passing the sample through Whatman filter paper (No.1), the extract was evaporated in a rotary evaporator under vacuum at 40 °C. Then, liquid-liquid extraction was performed using 10 ml of an n-hexane and NaOH solution (1:1). After the organic phase separation and condensation process, the drugs were analysed using an HPLC system.

2.4. HPLC analysis condition

COD and APAP were determined in extracted samples by reversed-phase C18 (Shim-pack HRC – ODS; Shimadzu company). The wavelength of the detector was set at 280 nm and 245 nm for the analysis of COD and APAP, respectively. For APAP analysis, the isocratic mobile phase was prepared with 160 ml water, 40 ml acetonitrile (pH = 3) and APAP retention time was determined at 5.8 min. The methanol/ acetic acid solution (9:1) was used for COD analysis as mobile phase and COD retention time was determined 4.3 min. The flow rate and column temperature were set at 1 ml/min and 40 °C respectively.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the ratio of S/N = 3 and S/N = 10, respectively.[6] In addition, the recovery experiments were performed by spiking 100 ng/ml of COD and 100,000 ng/ml of APAP into herbal samples.

3. Results and discussion

In the present study, the analytical methods for APAP and COD were validated separately. The calibration curves in the r^2 indexes were within acceptable linear ranges. The calculated LOD and LOQ contents for the analysed COD and APAP also showed a good performance at low statutory limits. In addition, the results of the recovery test were within the acceptable range, indicating the accuracy and precision of the analytical methods used in the study (Table 1).

APAP/COD were detected in 15% of the samples in the range of 38900–165200 ng/g for APAP and 31.1–603.3 ng/g for COD. The average APAP and COD concentrations were 94,500 ± 55,800 ng/g and 366.7 ± 258.6 ng/g, respectively. In the previous study, Joo Kim et al. (2014) reported the presence of non-steroidal anti-inflammatory drugs (NSAIDs) and APAP in some foods and dietary supplements. Meanwhile, Ibuprofen was the most commonly used medicine in about one-third of the adulterated samples.[3]

Overall, COD and other derivatives of morphine are natural components in some plants. For instance, significant amounts of COD are found in opium prepared from the unripe pods of *Papaver somniferum*.[7] However, the presence of synthetic drugs, such as APAP, in combination with COD may confirm the occurrence of adulteration in herbal medicines.

APAP is available in two forms: alone and as an APAP/COD combination. In Iran, the amount of COD used in APAP/COD tablets ranges from 8 to 20 mg. APAP is also available in 100-, 325- and 500-mg tablets. In this study, the average ratio of APAP/COD was 257.7 units, which is much higher than the ratio of this medicine in drug markets (about 15–30 unit). This discrepancy may be explained, at least in part, by the use of APAP alone, along with its COD form, which increases the ratio of APAP to COD.

The health risk in the Hamadan population was calculated based on the ratio of the estimated APAP and/or COD exposure through the consumption of medicinal herbs and/or herbal supplements. The therapeutic dose of oral APAP in adults is 325–650 mg every 4–6 h with a maximum of 4000 mg/day. In children, the therapeutic dose is 10–15 mg/kg every 4–6 h with a maximum dose of 50–75 mg/kg/day.[8] In addition, the maximum daily dose of COD was estimated to be 240 mg.[9] Based on the reports collected from Hamadan's apothecaries, the recommended dosage of herbal medicines by druggists is estimated at 12 g/day. If the maximum APAP and COD were considered in the exposure assessment calculations, consuming herbal medicines each day may not have toxic effects on human (Table 2).

4. Conclusion

Taken collectively, the presence of synthetic drugs, such as APAP/COD, are illegal in herbal medicines. However, the findings of this study suggest that the APAP and COD concentrations in these products may be considered safe in humans with a body weight of at least 60 kg if the consumption of these supplements is 12 g/day.

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