The Detection of Tramadol in Honey

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Abstract:
Tramadol is a centrally acting analgesic selective for the μ-opioid receptor with antidepressant and dopamine releasing actions. In Egypt, dependence on and abuse of tramadol presents a challenging health problem and the drug is included in the narcotic analgesic schedule III. In this study, a sample of commercially available honey was brought to the laboratory of narcotics and poisons on the suspicion of withdrawal symptoms upon discontinuation. The presence of tramadol was suspected and the sample was subjected to thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Liquid-liquid extraction was carried out using a mixture of chloroform and isopropanol (4:1, v/v)(3:1) in an alkaline medium (pH 8.5). The re-constituted residue was spotted on a TLC plate along with standard tramadol and sprayed with Dragendorf’s reagent. Honey samples were found positive for tramadol. Determination of tramadol was then carried out using HPLC. Separation was achieved on ODS-reversed phase column (C18, 25 x 0.46 cm i.d.5 µm), the mobile phase consisted of water/acetonitrile/triethylamine 40/60/0.1 v/v, and was delivered at a flow rate of 2 ml/min. UV detection was performed at 280 nm, and the injection volume was 20µL. HPLC indicated the presence of tramadol HCl in honey. The concentration of tramadol in the sample was determined by an external standard method using peak area. The concentration of tramadol in the sample was then found to be 36.5 mg/ml. The present study is the first which describes the identification of tramadol in commercial honey.

Keywords: honey; tramadol; high-performance liquid chromatography

Introduction:
Tramadol is a centrally-acting analgesic with opioid and non-opioid properties. In clinical practice, it is used worldwide for the prevention and treatment of moderate to severe pain in acute or chronic conditions [1]. This centrally acting analgesic is a synthetic 4-phenylpiperidine analog of codeine [2]. Tramadol is (1RS;2RS)-2-(dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol-hydrochloride [3]. The drug is an opioid agonist with selectivity for the μ-opioid receptor [4]. In addition to its actions on brain opioid receptors, tramadol also inhibits the reuptake of serotonin and noradrenaline [5,6]. Tramadol enhances dopamine release in
specific brain areas, including medulla oblongata, hypothalamus, corpus striatum, and nucleus accumbens, which may mediate reinforcing effects [6]. In humans, tramadol (350 mg) induced miosis and mixed subjective effects, increasing the rating of “good effects” and “liking” as well as the unpleasant subjective effects. Only the pleasant effects were partially attenuated with the opioid antagonist naltrexone [7]. In recent years, there has been an increasingly growing number of human studies and cases reports of tramadol dependence, abuse, and intoxication [8-11]. The present study describes the identification of tramadol in commercial honey.

Materials and methods:
A sample of labeled honey jar was brought to the laboratory of narcotics and poisons to be verified for the presence of tramadol HCl (the narcotic analgesic schedule III). There consumers of the honey suspected the presence of a substance of abuse because of feeling unwell upon discontinuing ingesting the honey. We suspected the presence of tramadol based on its widespread abuse in Egypt [12-15].

Preparation of standard solution
A stock solution of tramadol (1 mg/ml) was prepared in 10 ml volumetric flask with methanol and stored at 4C. Working solutions for calibration curve were prepared using methanol (HPLC grade).

Preparation of sample
A sample of labeled honey jar was submitted to the laboratory of narcotics and poisons to be verified for the presence of tramadol HCl (the narcotic analgesic schedule III). First, the sample was examined for its characteristics (viscosity, color, and odor) and it showed normal features of honey.

Liquid-liquid extraction was carried out using a mixture of chloroform and isopropanol (4:1, v/v) (3:1) in an alkaline medium (pH 8.5). Briefly, 100 ml of honey was diluted with 20 ml of distilled water and alkalinized by sodium hydroxide (10N) to pH 8.5(10), then well-shaken with 50 ml of chloroform mixture for 10 min and repeated three times. The final organic phase was transferred to a flask and evaporated under a gentle stream of nitrogen then the residue was re-constituted in 1ml (10 ml) of methanol (HPLC grade). The re-constituted residue was spotted on TLC plate along with standard tramadol and sprayed with Dragendorff’s reagent.

A 20 µL aliquot of the solution was injected into the HPLC system.

Results and discussion
Thin layer chromatography
The spotted sample had the same hRf value of the standard and gave the same orange-red color with Dragendorff’s spray reagent.

High-performance liquid chromatography (HPLC)
Determination of tramadol was carried out using HPLC, Agilent technologies 1100 series equipped with a quaternary pump (Quat pump, G 131A model). Separation was achieved on ODS-reversed phase column (C18, 25 x 0.46 cm i.d.5 µm). The mobile phase consisted of water/acetonitrile/triethylamine 40/60/0.1 v/v and was delivered at a flow rate of 2 ml/min. UV detection was performed at 280 nm, and the injection volume was 20µL.

The concentration of tramadol in the sample was determined by an external standard method using peak area. Serial dilutions of the standard were injected and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentration of tramadol in the sample was then obtained from the curve and was found to be 36.5 mg/ml.
Discussion

In the present study, we describe the first identification of tramadol in a sample of commercially available honey. The sample was brought to our laboratory suspecting the presence of a drug of abuse because of the awareness of the consumers of withdrawal symptoms when discontinuing ingesting the honey. Since abuse of and dependence on tramadol is a widespread health problem in Egypt [12-15], the presence of tramadol was initially suspected and later proved to be the case.

The abuse of tramadol is a growing health concern worldwide [8-11]. The drug is a potent centrally-acting analgesic owing to a selective action on μ-opioid receptors [1]. It is used to alleviate moderate-to-severe pain [4]. Side effects of the drug include nausea, vomiting, dizziness, drowsiness, and orthostatic hypotension [16]. In addition, severe hyponatraemia [17] and generalized tonic-clonic seizures have also been described with high doses of tramadol [10]. Overdose can cause ventricular tachycardia and, cardiac arrest in asystole with refractory shock [18, 19].

Tramadol dependence does occur within the recommended therapeutic doses and could be severe in those who are susceptible [20]. Even after disease regression, the patient usually continues to take the drug because of a feeling of well-being [21, 22]. The drug inhibits neuronal reuptake of noradrenaline and serotonin thereby enhancing monoaminergic neurotransmission [5,6]. The agent is thus likely to have antidepressant properties and this might be one reason which accounts for the continued intake of tramadol because of a mood enhancing effect (to improve mood) despite pain regression [23, 24]. Withdrawal symptoms occur after tramadol discontinuation. Mostly, withdrawal symptoms are those of classical opioid withdrawal (eg., abdominal cramps, anxiety, depression). However, atypical withdrawal symptoms might occur including hallucinations, delusions, paranoia, panic attacks, and confusion [25] or even seizures [26].
The importance of the present finding of tramadol in honey also derives from the ability of the drug to cause serotonin-like syndrome if taken with other drugs that increase serotonin activity [27]. The latter is a life-threatening condition resulting from excessive stimulation of serotonergic neurotransmission with disorientation, agitation, restlessness, tremors, seizures, coma, cardiac arrhythmia, and hypertension [28]. Serotonin syndrome has been reported following treatment with tramadol and either selective serotonin-reuptake inhibitors [29] or the atypical antipsychotic ziprasidone [30].

**Conclusions**

The present study describes the identification of tramadol in commercial honey. This finding should alarm authorities on the ways used to introduce this drug with addiction liability into food.

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**Declaration**

On behalf of all Co-Authors, the corresponding Author, states that this research work is original and has not been published in whole or in part elsewhere.

**Authorship (author(s) contribution or attribution)**

All Authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, agree to its content and submission to ScholReps and approved the version to be published.

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