Full Length Research Paper

Cumulation of *Tabernanthe Iboga* alkaloid and its metabolite in organs of mice

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The accumulation of *Tabernanthe Iboga* alkaloid ibogaine and its metabolite noribogaine in liver, kidney, spleen, heart, brain and muscle after administration directly into the stomach in white laboratory mice were studied. For this purpose a high-performance liquid chromatography (HPLC) method with fluorescein as internal standart were chosen. Solid phase extraction (SPE) method was employed to isolate the tested substances from the internal organs. Separation of the analytes was performed on a reversed-phase XTerra RP18 analytical column (3×150 mm, particle size 3.5 µm) using a gradient, the mobile phase consisted of a mixture acetonitril and 0.1% TFA in water. The total run cycle was 25 min. The flow rate was 0.4 ml/min, and injection volume was 10 µl. The excitation wavelength was set at 230 nm and emission wavelength at 336 nm. Excitation wavelength of internal standard (fluoresceine) was set at 440 nm and emission wavelength at 514 nm. Experiment demonstrated that highest concentration of ibogaine and noribogaine have been obtained in spleen and the lower one – in muscle of the animal after the direct administration into the mice stomach.

Key words: *Tabernanthe Iboga*, ibogaine, noribogaine, solid phase extraction, distribution in mice organs, HPLC.
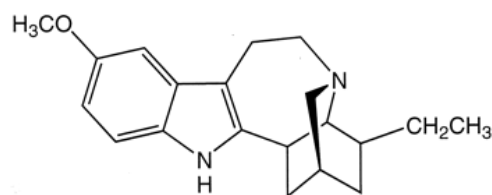
INTRODUCTION

Unabated drinking of alcohol remains a serious problem in the world. This induces further and deeper investigation of the alkaloid ibogaine, which is able to relieve the alcohol addiction (Rezvani et al., 1995) and has certain anti-drug effect (Lotsof, 1995, 1991; Sisko, 1993).

Tabernanthe Iboga of *Apocynaceae* family is a perennial rainforest shrub, growing in West and Central Africa (Kazlauskas et al., 2004). The active principle in iboga is ibogaine, the highest concentration being found in the root (Cousins and Huffman, 2002). For over a century small doses of *T. Iboga* extracts have been used as stimulants, big doses – as hallucinogens. There is evidence to state that even single dose of ibogaine relieves attraction to opiates and cocaine and attenuates the symptoms of interrupted intake of opiates (Sheppard,
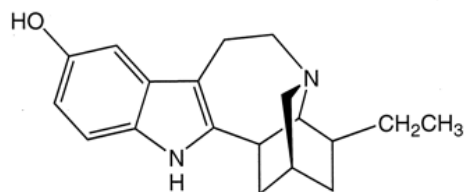
1994; Alper et al., 1999). The plant is employed in traditional African medicine, along with Tabernaemontana species, for manic depression, leprosy and as an aphrodisiac (Cousins and Huffman, 2002). Ibogaine (12-methoxyibogamine) causes oxidation of serotonine and expedites that of catecholamines (Taylor, 1957). Its effect is determined by complex CNS interaction of many neurotransmitory systems. Iboga alkaloids are reported to reduce the self-administration of morphine, cocaine, amphetamine and to diminish dopamine efflux in the nucleus accumbens, which is regarded as a correlate of drug salience, in response to opioids or nicotine (Alper et al., 2008).

Study in 2010 showed that ibogaine inhibited human P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) (Tournier et al., 2010). Ibogaine's mechanism of action is still not clear enough. The identified antagonistic activity of ibogaine on N-methyl-D-aspartate receptors as well as its agonist activity on opioid receptors can be regarded as a possible mechanism of anti-addictive action (Popik et al., 1995).

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Figure 1. Structure of Ibogaine and Noribogaine.

Ibogaine is metabolized in the human body by cytochrome P4502D6 (CYP2D6) Mash et al. (2000) to the major metabolite noribogaine (12-hydroxyibogamine) Figure 1. This enzyme showed the highest activity toward the formation of noribogaine, followed by CYP2C9 and CYP3A4 (Obach et al., 1998). Experiments with rats have established pharmacological activity of noribogaine and its effect being similar to that of ibogaine: relieved attraction to morphine and cocaine, reduced locomotoric activity of morphine (Glick et al., 1996). The comparison of ibogaine and noribogaine toxicity for mice was performed. It was detected that the latter is 2.4 times lower than that of ibogaine. Noribogaine appears less likely to produce the adverse effects associated with ibogaine (i.e. tremors and stress-axis activation), suggesting that the metabolite may be a safer alternative for medication development (Baumann et al., 2000).

General pharmacological actions of ibogaine are reported. These actions seem to depend on both the dose and setting. The distribution of ibogaine (40 mg/kg) in plasma, brain, kidney, liver and fat after ip and sc administration in rats are studied (Hough et al., 1996). Thus, the aim of the present experiment was to establish distribution of ibogaine and noribogaine in organs of white laboratory mice then tested substances were administered directly into the stomach via the stomach tube.

EXPERIMENTAL

Materials and reagents

Ibogaine hydrochloride, fluoresceine sodium salt (internal standart),

HPLC - grade acetonitrile, trifluoroacetic acid (TFA) and acetic acid were purchased from Sigma (St. Louis, MO, USA). Noribogaine base was kindly supplied by Reform Italia (Endine, Italia). Purified water was generated by a Milli-Q reagent water system (Millipore corporation, 106 Bedford, MA).

Animals

The white laboratory mice were used in the experiments. All experiments performed according to the Republic of Lithuania Law on the Care, Keeping and Use of animals (License of State Veterinary Service for working with laboratory animals No 0172). Different concentration suspensions of ibogaine and noribogaine were administered separately and directly into the mice stomach via the stomach tube. In the fullness of time mice were terminated and internal organs (liver, kidney, spleen, heart, brain and muscle) were taken and frozen at -40°C temperature.

Preparation of standard and working solutions

Standard solutions were prepared by dissolving accurately weighed quantities of ibogaine hydrochloride, noribogaine or internal standart. Ibogaine hydrochloride or fluoresceine standart solutions were prepared with purified water to give solutions containing 44.4 and 40.1 mg/L of each compound. The noribogaine standart solution was prepared in methanol at concentrations of 40.8 mg/L. Solutions were stored at 4°C.

Stock solutions of ibogaine and noribogaine were diluted daily in purified water to obtain 11 working standards ranging from 0.0444 to 4.44 mg/L and 0.048 to 4.08 mg/L respectively. The stock solution of fluoresceine was diluted 2 fold (20.1 mg/L) in purified water before use.

Sample preparation

Samples were subjected to a solid phase extraction (SPE) for the removal of proteins and interfering components. SPE Oasis HLB cartridges (30 mg of sorbent, average particle diameter 30 μm, supplied by Waters, Milford, USA) were used to establish the tested substances from the internal organs of mice. The cartridges were pre-washed with 1 ml of methanol followed by 1 ml of purified water.

The organ was washed twice for 30 s in 0.9% sodium chloride to limit blood contamination, powdered in a mortar. An aliquot of 50 to 350 mg, depending on the organ, was accurately weighed in a polypropylene tube, then carefully mixed for 20 s with 300 μl of blank human plasma, incubated at 4°C temperature (Kontrimaviciute et al., 2006) for 24 h and then 0.5 μl 0.1% acetic acid and 20 μl of internal standard (20.1 mg/L) were added. The mixture was vortex mixed and centrifuged for 20 min 6037 × g. The supernatant was loaded onto the conditioned extraction column under a light vacuum (approximately 86 kPa). The extraction column was washed with 2 x 1 ml of purified water and then was dried for 2 min by vacuum aspiration. The analytes were eluted from the column with 2 × 1 ml of methanol under a light vacuum. The eluate was dried at 40°C under a stream of nitrog en. The dried residue was reconstituted in 100 μl of the mobile phase (15% of acetonitril and 85 of 0.1% trifluoracetic acid (TFA) solution). An aliquot of 10 μl was injected into the HPLC system.

High-performance liquid chromatography (HPLC) analysis

HPLC analysis was performed using a model Waters 2695 chromatography system (Waters, Milford, USA) equipped with Waters 474 fluorescent detector. For separation, XTerra RP18 3.5
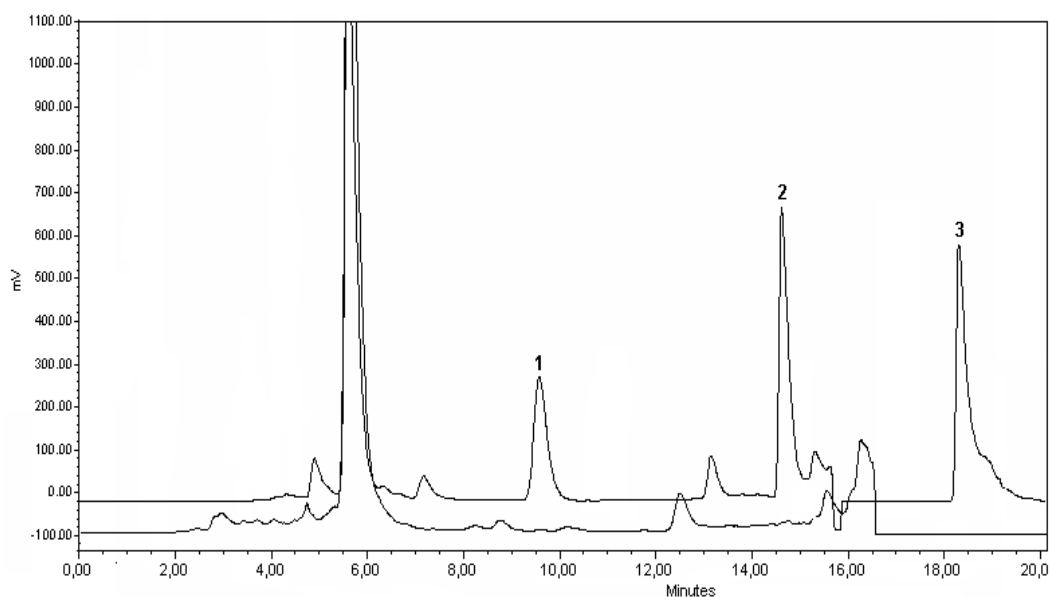
Figure 2. Chromatograms of blank human plasma and blank human plasma spiked with noribogaine, ibogaine and fluoresceine. Peak 1 is noribogaine, peak 2 is ibogaine, peak 3 is internal standard.

µm column (3×150 mm) and guard-precolumn 3.9×20 mm were used. The mobile phase consisted of a mixture acetonitril (eliuent A) and 0.1% TFA in water (solvent B). The elution profile was as follows: 0 to 7 min; 15% A (85% B), 20 min; 80% A (20% B), 22 min; 100% A (0% B), 25 min; 15% A (85% B). The flow rate was 0.4 ml/min, and injection volume was 10 µl. The excitation wavelength was set at 230 nm and emission wavelength at 336 nm. Excitation wavelength of internal standard (fluoresceine) was set at 440 nm and emission wavelength at 514 nm. Under the given conditions the noribogaine retention time was about 9.4 min, ibogaine; 15.2 min.

Validation procedure

The HPLC method validation parameters in our study were specificity, precision (between-day precision), linearity and lower limit of quantitation.

The specificity of the method was investigated by analyzing three different batches of blank human plasma samples. The retention times of endogenous compounds in the matrix were compared with that of the compounds of interest.

Between-day precision of the assay was assessed by performing replicate analyses of quality control samples in plasma. The procedure was repeated on 6 different days on the same spiked standards to determine relative standard deviations as precision criteria.

Lower limit of quantitation (LLOQ) estimated on samples in plasma. The signal-to-noise ratio was calculated according European Pharmacopoeia.

RESULTS

Validation results

Specificity

Chromatographic peaks of different heights and areas were indicated testing blank human plasma. This shows that the SPE used under these conditions did not guarantee complete eliminate of impurities in our study. However, these peaks did not impede the identification of the tested materials as the retention time for noribogaine is about 9.4 min, ibogaine; 15.2 min and fluoresceine; 18.6 min (Figures 2 and 3), where any peaks from matrix were observed. The peak at 11.5 min was observed on chromatograms of all organs. This supposedly is produced by one more Ibogaine metabolite which was not identified.

Precision and lower limit of quantitation (LLOQ)

Precision procedure was repeated on 6 different days on the same spiked standards. RSD were calculated: it was 5,1% for noribogaine and 6,9% for ibogaine. The LLOQs were 1.4 ng/ml for ibogaine and 2.15 ng/ml for noribogaine. These concentrations were chosen as the lowest concentrations of the standard calibration curves.

Linearity

The regression analysis between peak area ratios of ibogaine and noribogaine over the internal standard and plasma concentrations revealed that the method is linear and formula:

$y = a + bx$; where x is concentration, y is peak area.
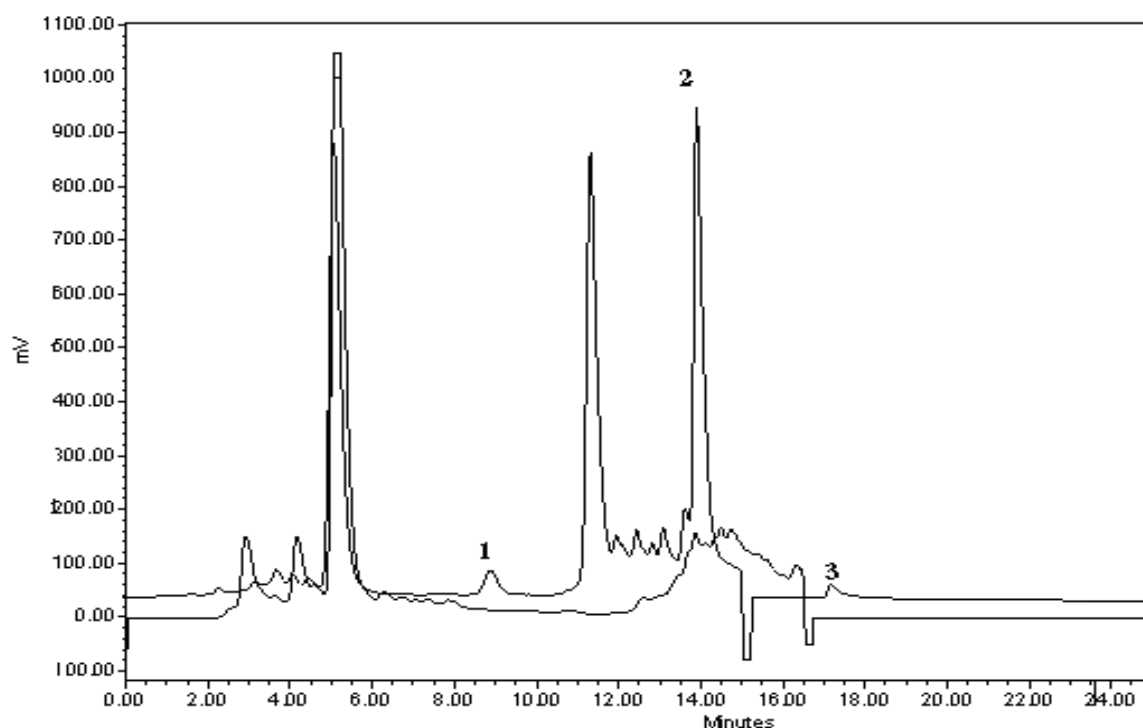
Human plasma standarts were prepared by appropriate

Figure 3. Chromatogram of control spleen and spleen spiked ibogaine. Peak 1 is noribogaine, peak 2 is ibogaine, peak 3 is internal standard.
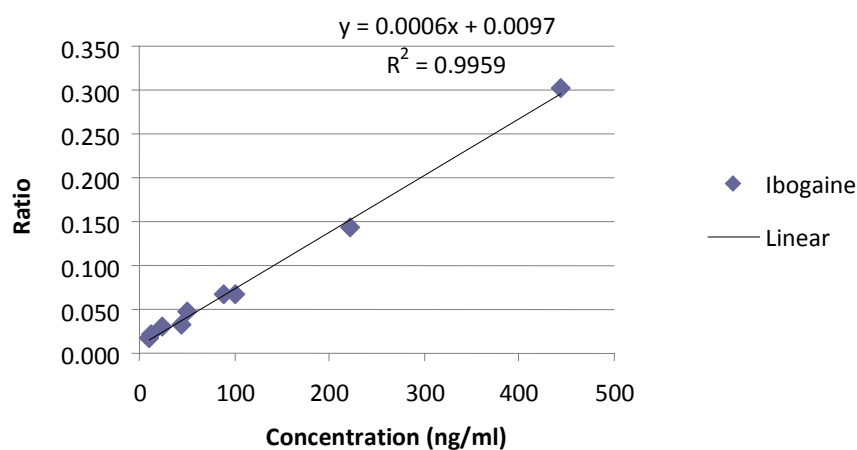

$$y = 0.0006x + 0.0097$$
$$R^2 = 0.9959$$

Figure 4. Calibration curve of ibogaine.

volumes of drug working solutions (20 µl) into 0.5 ml of drug free human plasma to produce a concentration series ranging from 1.4 to 444 ng/ml of ibogaine and 2.15 to 444 ng/ml of noribogaine. For each calbration curves, 9 concentrations were used. The correlation coefficients (r) for calibration curves were equal to or better than 0.98 (Figures 4 and 5). To evaluate the distribution of ibogaine and noribogaine in the organs of mice, the substance

concentration obtained from the calibration graph was recalculated for 1 mg on each organ of mouse.

Results from experiment

Experiment demonstrated that highest concentration of ibogaine and noribogaine have been obtained in spleen,
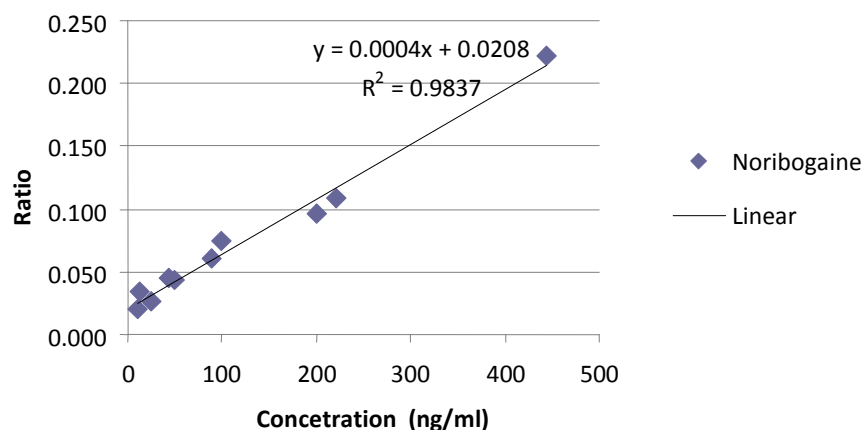
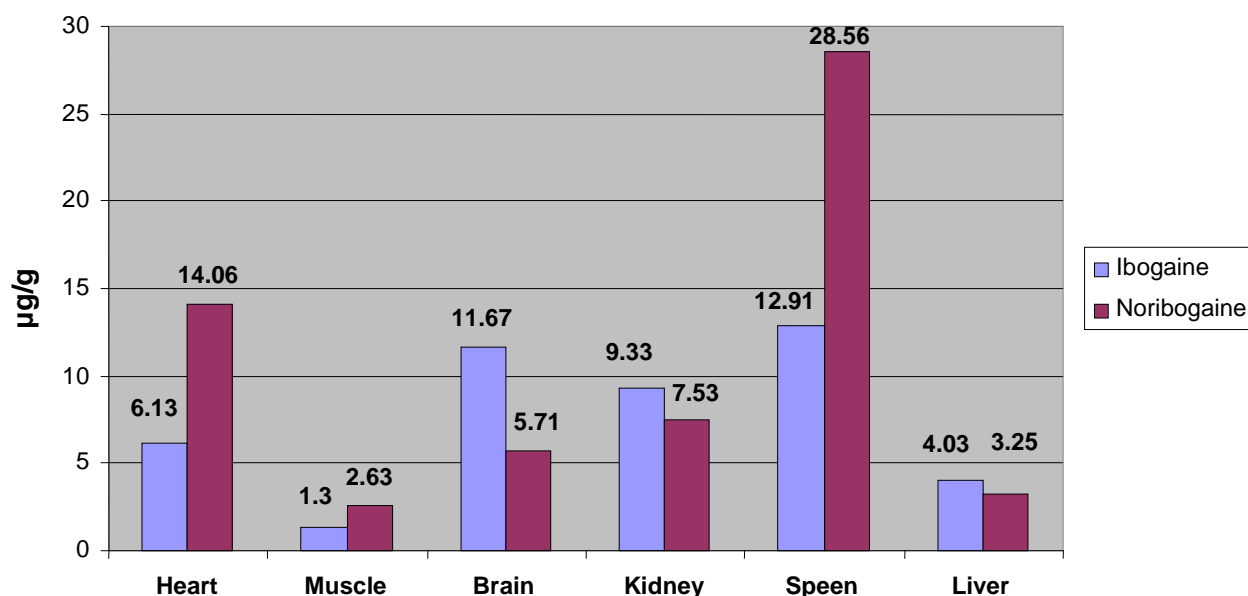**Figure 5.** Calibration curve of noribogaine.

Figure 6. Cumulation of Ibogaine and Noribogaine in the organs of mice.Ibogaine n=6; Noribogaine n=8

but not in liver. It was unexpected, because firstpass metabolism was not avoided. And the lower one – in muscle of the animal after the direct administration into the mice stomach (Figure 6).

DISCUSSION

Our results differ from the findings of Hough (Hough et al., 1996) who reported that the higer concentration of ibogaine and noribogaine were found in fat but similarly Kontrimaviciute (Kontrimaviciute et al., 2006), who the highest concentrations found in spleen, liver and lung after ingestion of root bark from the shrub *T. iboga.* The

reasons for such discrepancies are unclear but may be related to differences in experimental design and methods between studies. Furthermore, there is the possibility of species or strain differences in ibogaine metabolism (Mash et al., 1998). Gall- bladder is not present in organism of rats that were analyzed in previous studies therefore one stage of link of metabolism is missing; whereas metabolism of mice is considered more contiguous to human metabolism. This could be one of the main reason of discrepancies of results of our and previous studies.

Spleen is the organ, which cleans blood from harmful materials; therefore, it quickly accumulates these tested substances. As ibogaine is metabolized to its metabolite,

there was more noribogaine vs. ibogaine in spleen. Meanwhile, muscle tends to take the necessary materials transported by blood.

Based on the data presented in literature, after intra-peritoneal and subcutaneous injection of ibogaine in rats, the highest level of the substance is achieved in brain and adipose tissue after one hour after administration (Kontrimaviciute et al., 2006). The calculations conducted in our experiment show that concentration of tested substances tends to increase in brain, heart and muscles after 1 h after intra-gastrically administration. Therefore, further investigations are planned to be based on pharmacokinetic studies of ibogaine and noribogaine in the organs of mice (heart, muscle, kidney, brain, spleen and liver) after per os administration and to compare this parameters with i.p and i.v. parameters.

Conclusions

Ibogaine is being investigated currently for its putative "anti-addictive" properties, but the mechanism of action is still unresolved. However, it seems ibogaine and its metabolite could be used as a medications for treating drug dependence, but more studies required. The presented method is specific for the determination and estimation of concentrations of these substances in organs of mice.

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