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# The Effects of Herbs, Spices and Foodstuffs on Urine Screens for Drug Abuse by Immunoassay

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## ABSTRACT

Drug abusers are highly motivated to obtain negative results on urine drug abuse screens and may attempt to subvert the process by trying to adulterate their samples. One of the processes thought to make drug abusers escape a positive urine drug screen is the ingestion of different herbal and other foodstuff products along with drug intake. This project aimed to evaluate the effects of common traditional herbal drinks, spices and some foodstuffs in Jordan on producing false negative urine drug screens for opiates, amphetamines and cannabinoid with fluorescent polarizing immunoassay (FPIA). Thirty one products were divided into two groups;the first group'sproducts were extracted with methanol while the second group's were extracted with water. Urine drug free samples were spiked with each product or its extract in addition to a known concentration of opiates, amphetamines or cannabinoids. The spiked urine samples were analyzed using FPIA. It was found that none of the 31 products had modified the drug screens using FPIA to reduce or increase the level of the spiked drug in the urine sample. It was concluded that these products did not produce falsenegative results with urine drug screenings by means of FPIA as it is believed by drug abusers. However, such conclusions should be taken cautiously since thisis not an idealevaluation. It would be ideal to apply such a study on normal volunteers to evaluate the actual disposition of the ingested substances and the drugs as well as their interactions within the body, but it cannot be conducted due to ethical considerations.

**Keywords**: Urine, drug abusescreen, adulteration, immunoassay.

## INTRODUCTION

Currently, screening for substance use occurs in a number of different contexts, and it is estimated that over 120 million screens and tests are performed annually.[1]Screening for drugs of abuse might have legal consequences thus positive results necessitate confirmation by unequivocal procedures.[2] Immunoassays are simple to use and allow relatively fast screens for a large number of samples once the reagents are developed and optimized. Also, the availability of various commercial kits and instruments facilitates the versatility of immunoassays in meeting the specific needs of drug screens .[3]The TDx system (Abbot Laboratories) utilizes FPIA for the detection of drugabuse in a biological matrix and has been commonly used in forensic toxicology.[4, 5]Immunoassay based drug screens are prone to interferences that might cause either falsenegative or falsepositive results, which necessitates further confirmatory analysis.[3, 6-12] Urine specimens are sufficient for most drug screen applications; however, they might contain interfering substances coming from either endogenous or exogenous sources which have the potential to change the results.[3] Depending on the disease or physical state of an individual, a urine sample may contain different components that can affect the result of a drug screen. Endogenous interferences include proteinurea, glucose, bilirubin, nitrates, *p*H, ketones,

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blood, leukocytes, bacteria, fungi and others.[3] Some reports showed the presence of different concentrations of ethanol in the urine of diabetic patients due to the fermentation of glucose in the sample upon prolonged storage.[13, 14] Exogenous interferences might include drugs, drug metabolites, herbal products, foods and vitamins. Most manufacturers of immunoassay kits provide a list of drugs that are expected to cross react to the antibodies in their products. Several studies concerning a wide range of medications and potential false positive urine drug screens havebeen published in the literature suggesting that various drugs and/or their metabolites interfere with an immunoassay and might produce true positive results in drugabuse screening such as ingesting poppy seed cakes or drinking Health Inca or Mate de Coca tea.[15-20]Adulteration hasoften been performed by drug abusers to mask the presence of an illicit drug and thus get false negative results for urine drug screening. They tend to tamper with a urine sample by *in vivo* adulterationwiththe ingestion of a substance prior to urination, or *in vitro* adulteration by adding a substance to a specimen post urination as well as sample substitution when a drug negative specimen is substituted for a potential drug positive specimen. The primary mechanism of action for *in vivo* adulteration is dilution and excretion.[21]Even the simple action ofexcessive water consumption can effectively dilute a urine specimen to cause false negative results.[22]Other measures can, however,follow for proper interpretation such as measuring urine specific gravity and creatinine levels which are expected to drop significantly indicating diluted specimens. The consumption of dietary supplements and some foodstuffs might interfere with urine drug screening by altering urinary *p*H or changing urine color. Most manufacturers of drug immunoassays specify that urine *p*H must be within a given range to obtain correct results.[3]Thus,the alterationof urine *p*H, outside the manufacturer's specified limits, could result in a false negative screen.

This project is a pilot study that aimed to evaluate the effect of common traditional herbal drinks, spices and some foodstuffs commonly used in Jordan on urine drug screens by analyzing urine samples on an Abbot TDx analyzer (FPIA) for opiates, amphetamines and cannabinoid.

**Materials and Methods**

Thirty one products including a variety of common traditional herbs, spices vegetables and fruits were prepared in two main groups according to the method of extraction used, and three products were studied in both groups. The common and botanical names of these products are listed in table I for group (I) and table II for group (II). Analyses wereperformed using FPIA with anAbbot TDx analyzer.

Group (I) included 20 different plants with the majority being herbs. The products were first dried and 200mg were steeped in 10ml methanol to prepare 20mg/ml methanolic stock solutions. A working solution for each product was prepared by diluting the stock with theTDx buffer to reach a concentration of 100µl/ml. Urine positive for cannabinoids, opiates, and amphetamines (12.5ng/ ml, 75ng/ ml, and200ng/ml,respectively) were used for preparing 1:10 dilutions of positive urine in working solutions and these dilutions were applied for FPIA analysis.

Group (II) included 14 plant items, where ten of them were studied as fresh plants and four as dry products. Fresh lemon, orange, and grapefruit were pressed, and the juice was filtered.Then the filtrates were directly diluted with theTDx buffer to prepare 5mg/ml working solutions. Fresh tomato, garlic, onion, parsley, coriander, rocket, and chicory extracts were prepared by crushing the plants and filtrating, and then filtrates were diluted with the TDx buffer to reach a concentration of 5mg/ml working solutions. Finally, the four dry items(ginger, cumin, licorice and mustard) were directly added to the TDx buffer to prepare 10mg/ml working solutions. Positive urine samples used for group (I) products'analyses were also used for group (II) plants and the 1:10 dilutions of positive urine in plant working solutions were used for the FPIA analysis of each single product.

The Abbot TDx instrument was operated in accordance withthe operator's manual. Each assay of ananalysis of the prepared products included a pre-

calibration curve using six different calibrators as well as low, medium and high positive controls, which were provided by the manufacturer.

**RESULTS AND DISCUSSION**

Urine positive for cannabinoids, opiates and amphetamines and the positive controls provided with assay kits all showed positive reactions with acceptable concentrations. All working solutions of the prepared products for group (I) and group (II) were negative for cannabinoids, opiates and amphetamines. Also none of the spiked positive urine samples showed any decrease in the response or produced false negative results. Therefore, none of the 31 products studied showed any false negative or false positive interfering effect with a fluorescent polarizing immunoassay.

The concentrations of urine positive controls were prepared in accordance to the cutoff value for each drug, in a way that urine positive control concentrations were slightly exceeding cutoff values for increasing the accuracy of the analysis. The cutoff values were 10ng/ml, 25ng/ml and 100ng/ml for cannabinoids, opiates and amphetamines, respectively.Values below the cutoff levels are reported as negative, which can lead to false negative testing. It is therefore recommended for all laboratories to evaluate cutoff values for their specific patient populations.[23]

FPIA utilizes a known amount of tracer that competes with the free drug in the specimen for antibody binding. Increasing the concentration of the drug in the specimen that binds to the antibody leads to a greater amount of unbound tracer, which contributes to depolarization of the emitted light. Thus, FPIA is susceptible to interference from light scattering and endogenous fluorophores in biological samples and from tracer binding to sample matrix components.[24]Such interfering factors must be taken into consideration for each of the products analyzed in the study.

The Abbott TDx system was used forits usefulness in urine drug screening and ease of application including the stability of the calibration curves for 3–4 months, possibility of providing semi-quantitative results, and the ability to process urgent samples.[25]

In contrast to this study, Winek C. et al.[26]evaluated the validity of claims among drug abusers that some herbal drinks interfere with urine drug testing and yielded false positive results. The *in vitro* effects of fifty herbal drinks were assessed by directly adding them to samples tested by FPIA and thin layer chromatography (TLC) assays. Four products that were included in this study were tested by the Winek et al. study and these were peppermint, thyme, caraway and licorice. It was concluded that none of the herbs screened showed any interference with TLC or FPIA.[26] However, both studies evaluated the *in vitro* effect of the products' extracts added directly to urine, but did not examine any potential interference metabolites that might be produced by *in vivo* use of such products. Only limited research on *in vivo* adulteration exists, partly because of the ethical considerations involved in performing such research on human subjects.[21]

Furthermore,the diuretic effect of such plants along with the excessive consumption of water may dilute the drug in urine below a screening cutoff, as suggested by some in the literature,which has to be considered.[22] However, Goldenseal (organ root) has been reported to create a false negative interfering effect with tetrahydrocannabinol (THC) positive urine samples analyzed by means of FPIA,[27] RIA,[28] and EIA[29]. Another study showed that more than94% of THC-acid was destroyed within 48h of *in vitro* exposure to examined oxidizing adulterants and extracts from red radish, horseradish, Japanese radish and black mustard seeds.[30]

Finally, it is worthy to say that urine drug abuse screens are valuable tools in health care, the workplace, and other settings. Accurate interpretation of the validity and reliability of these tools is critical for making decisions that will ultimately have social and legal consequences [31]

In conclusion, it was found that none of the 31 products modified the drug screen for cannabinoids, opiates and amphetamines using FPIA to reduce or increase the level of the spiked drug in the urine sample and the tested products did not produce false negative results with urine drug screen for cannabinoids, opiates

and amphetamines by means of FPIA as it is believed by drug abusers. However, these conclusions should be viewedcautiously and it ishighly recommended to apply more studies in the field of *in vivo* adulteration to evaluate drug abuse testing utilizing immunoassay on normal volunteers and expand the study to include other possible dietary products the might produce false negative results. Applying *in vivo* studies will evaluate the actual disposition of the ingested substances and drugs within the body, but such studies require careful ethical considerations.

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**Table (I)**

| **Common name** | **Botanical name** |
|---|---|
| Syrian bear's breeches | *Acanthus syriacus* |
| Fenugreek | *Trigonella foenum* |
| Garlic | *Allium sativum* |
| Peppermint | *Mentha piperita* |
| Nutmeg | *Fragrans myristica* |
| Carrot | *Daucus carota* |
| Radish | *Raphanus sativus* |
| Carnation | *Eugenia caryophyllata* |
| Parsley | *Petroselinum crispium* |
| Anise | *Pimpinella anisum* |
| Cinnamon | *Cinnamomum zylanium* |
| Thyme | *Thymus vulgaris* |
| Coriander | *Coriandrum sativum* |
| Gundelia | *Gundelia toumefortii* |
| Hermon giant fennel | *Ferula hermonis* |
| Cabbage | *Brassica oleracea L.var. capitata* |
| Caraway | *Carum carivi* |
| Lemon balm | *Melissa officinalis* |
| Black cumin | *Najella sativa* |
| Fennel | *Foeniculum vulgare* |

**Table (II)**

| **Common name** | **Botanical name** |
|---|---|
| Tomato | *Lycopersicon lycopersicum* |
| Grapefruit | *Citrus Paradisi* |
| Lemon | *Citrus Limonum* |
| Orange | *Citrus aurantium* |
| Garlic | *Allium sativum* |
| Onion | *Allium cepa* |
| Parsley | *Petroselinum crispium* |
| Coriander | *Coriandrum sativum* |
| Rocket | *Eruca veriscaria* |
| Licorice | *Glycyrrhiza glabra* |
| Cumin | *Cuminum cyminum* |
| Ginger | *Zingiber officinale* |
| Chicory | *Cichorium intybus* |

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# تأثير الأعشاب والبهارات وبعض أنواع الغذاء على الكشف البولي المناعي لأدوية الإدمان

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## ملخـص

يعمد المتعاطون لأدوية الإدمان للحصول على نتائج سلبية للكشف البولي على أدوية الإدمان، وذلك بمحاولة الإخلال بالفحص عن طريق غش العينة. وإحدى هذه الطرق أن يتناول المتعاطي العقار مع بعض المنتجات العشبية أو بعض الأغذية. يهدف هذا البحث إلى تقييم تأثير مشروبات الإعشاب التقليدية الشائعة في الأردن أو البهارات أو بعض المنتجات الغذائية على احتمالية إعطاء نتيجة سلبية للكشف البولي لمواد الأفيون والفيتامينات والقنب، باستخدام التحليل المناعي الذي يعتمد على الاستقطاب الفلوري. تم اختيار واحد وثلاثين منتج وتم تقسيمها إلى مجموعتين، حيث تم استخلاص المجموعة الأولى بالميثانول و استخلاص المجموعة الثانية بالماء. تم تحضير عينات بول تجريبية بإضافة كميات من مواد الأفيون والامفيتامينات والقنب بتركيز معلوم ، إلى عينات بول خالية من هذه المواد. وبعد ذلك تم تحليل هذه العينات باستخدام التحليل المناعي وبالاستقطاب الفلوري. وقد وجد انه لم يحدث أي منتج من المواد الواحد والثلاثين أية زيادة أو نقصان في مستوى الاستجابة. وقد استنتج أن هذه العينات لم تعط نتيجة سلبية خاطئة باستخدام الكشف البولي المناعي حسب اعتقاد المتعاطين ، ولكن ينصح بمراعاة الحذر عند الأخذ بهذا الاستنتاج بشكل مطلق، حيث أن النموذج المثالي هو أن يتم إجراء مثل هذه التجارب على متطوعين طبيعيين، ليتم تقييم تأثير حركيات العقار والمواد وتداخلاتها ونواتجها داخل الجسم، على الكشف البولي المناعي، ولكن ولأسباب أخلاقية، يصعب تطبيق وإجراء مثل هذه التجارب في الحياة العملية.

الكلمـات الدالـة: الكشف البولي المناعي، غش العينة، الإدمان.

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