The Effect of Oral Contraceptive Pills on the Detection of Pregabalin and Diazepam in Urine: Animal-Based Model

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A B S T R A C T

Background: Oral contraceptive pills (OCPs) are usually used for childbirth control. However, some studies showed unforeseen uses for them, including trials to adulterate the results of illicit drug tests.

Objective: We examined the effects of OCPs on masking the appearance of Pregabalin and diazepam in a urine drug screening kit. As well, the behavioral consequences of Pregabalin and diazepam with or without OCP were examined using rotarod and hot plate tests.

Methods: A total of 60 male Wester Albino rats were randomly divided into six groups: (a) a control group (untreated), (b) a group given pregabalin alone, (c) a group given diazepam alone, (d) a group given OCP (Microgynon® (Levonorgestrel/Ethinylestradiol)), (e) a group given pregabalin with OCP, and finally (f) a group given diazepam with OCP. A urine drug screening kit was used to determine the presence and absence of illicit drugs. Rotarod and hot plate tests were also used to assess the impact of OCP use on behavior among the tested groups.

Results: Urine screening test showed a confirmed drug detection in Pregabalin group, Diazepam group, and Pregabalin/Microgynon group, while Diazepam/Microgynon group showed uncertain presence or absence of diazepam in the urine. Rotarod and hot plate tests showed that Microgynon did not significantly affect the pregabalin-treated group, whereas a slight difference was observed in the diazepam-treated group.

Conclusion: This study demonstrated that OCPs did not affect the detection of illicit drugs in the urine immunoassay tests; however, they may contribute to invalid results among specific abused drugs. Future studies are needed to understand the rationale behind the improper use of OCPs to mask illicit drug detection. Serious educational campaigns are also necessary to highlight the negative consequences of OCP intake on men’s health.

Introduction

In the past decade, non-therapeutic drug use, which is determined by drug abuse, has grown significantly (1). Drug abuse is characterized by the harmful consumption of substances beyond the approved medical practice and guidelines. This includes self-medicating with higher dosages, longer durations than what is recommended, intoxicated motivations, and situations where risks outweigh benefits (2, 3).

Methods and materials

The used OCPs and Drugs

Nervica+ (Pregabalin 75mg capsule, IOSWE medical, Jordan), Valium® (Diazepam 5mg tablet, Roche Holding AG, Switzerland), and Microgynon® (Levonorgestrel/Ethinylestradiol tablet, Bayer Wemar GmbH & Co KG, Germany).

Animals management

Male Wester Albino rats (n=60), aged 10- to 12-week-old, and weighing an average of 250 to 300 g, were inbred at the Applied Science Private University of Jordan (ASU), Jordan. Animals had free access to food and water in their Plexiglas cages (60 × 25 × 25 cm), and were kept on a controlled humidity (50% ± 3%), and temperature (23° ±2° C), with a 12/12-h light/dark cycle (lights turned on at 7:30 AM) throughout the duration of the experiment Figure 1.

Study settings

All animal procedures were accomplished in compliance with the regulations and guidelines of the Research and Ethical committee at the faculty of pharmacy – Applied Science University, Amman, Jordan, and according to the strict national and international regulations about laboratory animals care and use.
Figure 1. Scheme describing the study method

Ethical approval was obtained from the Institutional Research Board in the faculty of Pharmacy Applied Science Private University (Approval number: 2020-PHA-28). The lab experiments were conducted by Alaa Al-Banna.

Animal groups and treatments

As shown in Table 1, following a one-week acclimatization period, rats were assigned randomly to one of six groups: Group 1 (Control group, n=10), which received distilled water by oral gavage during the experiment (untreated group). Group 2 (Pregabalin group, n=10) received pregabalin treatment (60 mg/kg) by oral gavage once daily for 7 days. Group 3 (Diazepam group, n=10), received diazepam treatment (10 mg/kg) by oral gavage once daily for 12 days. Group 4 (Microgynon group, n=10) received Microgynon treatment (2.6 mg/kg) by oral gavage once daily for 7 days. Group 5 (Pregabalin/Microgynon group, n=10), which received Pregabalin treatment (60 mg/kg) by oral gavage for 7 days, and then a dose of Microgynon treatment (2.6 mg/kg) by oral gavage once daily for another 7 days. Group 6 (Diazepam/Microgynon group, n=10), which received Diazepam treatment (10 mg/kg) by oral gavage for 12 days once daily, and then a dose of Microgynon treatment (2.6 mg/kg) by oral gavage once daily for another 7 days.

Urine drug testing

Urine drug testing is commonly used in medical clinics to exclude substance-induced disorders, monitor medication adherence, and detect drugs in cases of overdose. Companies and governments also conduct drug testing to detect illicit drug use (27, 28). In the current experiment, collected urine samples were screened for illicit drugs or their metabolites using a 10-panel urine drug test kit (ALL TEST 10 Panel Drug Test Kit DOA-1104-KET, China). This drug testing kit is certified by the Food and Drug Administration. The kit can detect ten different drugs including cannabis, cocaine, amphetamine, methamphetamine, ecstasy, opiates, methadone, buprenorphine, benzodiazepines, and ketamine. Results were recorded as positive or negative based on the manufacturer’s cut-off values: cannabis (50 ng/mL), cocaine (300 ng/mL), amphetamine (1000 ng/mL), methamphetamine (1000 ng/mL), ecstasy (1000 ng/mL), opiates (2000 ng/mL), methadone (300 ng/mL), buprenorphine (10 ng/mL), benzodiazepines (500 ng/mL), and ketamine (1000 ng/mL). The bottom end of the test strips was immersed into urine samples and allowed to settle for five minutes before reading the results. The appearance of one line indicated positive drug detection, whereas the appearance of two clear lines indicated the absence of the drug in urine. In case one clear line and one pale line suggested that the result was unclear, the test should be repeated.

Samples collection

The rats were individually housed in metabolic cages (Techniplast, Italy), with stainless steel surface area, measuring 23 cm in diameter and 18 cm in height. Urine samples were collected and tested at intervals of 3, 5, 10, 24, 30, 40, 50, and 56 hours after administration of the last dose of each treatment in Group 1 (distilled water), Group 2 (Pregabalin), Group 3 (Diazepam), and Group 4 (Microgynon). While in Group 5, and Group 6, urine samples were collected and tested at intervals of 3, 5, 10, 22, and 30 hours after each dose of OCP (Microgynon) administration.

<table>
<thead>
<tr>
<th>Group name</th>
<th>Drug</th>
<th>Duration of intake per day and the dose</th>
<th>Details</th>
<th>Group name</th>
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<th>Duration of intake per day and the dose</th>
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<tbody>
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<td>Control group</td>
<td>Untreated</td>
<td>Non</td>
<td>No drugs or vehicles</td>
<td>Pregabalin group</td>
<td>Pregabalin</td>
<td>60 mg/kg for 7 days</td>
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<td>Diazepam group</td>
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<td>Microgynon group</td>
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<td>Pregabalin/Microgynon group</td>
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<td>60 mg/kg for 7 days followed by Microgynon for another 7 days</td>
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<td>Diazepam/Microgynon group</td>
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<td>10 mg/kg for 5 days followed by 2.6 mg/kg for another 7 days</td>
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Table 1. Details of illicit drug administration
**Motor Activity assessment**

The rotarod test is usually used to assess motor activity behavior and to determine an animal’s ability to balance on a rotating rod (29). In this test, rats were initially pre-trained on an automated one-lane rotarod (MUROMATCHI KIKAI Co., LTD., MK-630B, Tokyo, Japan), which was set on accelerating speed ranging from 4 to 40 rpm over 300 seconds period (30, 31). To alleviate stress and exhaustion, animals were given a minimum of 20 minutes break between each speed. The duration each animal remained on the rod, recorded as latency to fall, was automatically documented using a switch under the bottom of the rotating drum. This test was carried out during the morning.

**Hot-plate test**

The hot plate test is a behavioral model of nociception that is often used to screen for pain reliever effects, which are typically measured by changes in the nociceptive threshold (32, 33). The hot-plate apparatus (MUROMATCHI KIKAI CO., LTD, MK 350D, Tokyo, Japan) was maintained at a temperature of 50 ± 0.5 °C. Rats were placed within an acrylic cylinder (20 cm in diameter) on a heated surface, and the time (in seconds) was started by a blinded investigator until the rat exhibited a sensory response, such as paw-licking, paw-lifting, or jumping (34), within a maximum delay of 60 seconds, to avoid tissue damage. At the end of each session, the hot plate was cleaned with water and allowed to dry before the next session was started. This test was carried out during the morning.

**Statistical analysis**

The data was presented as means and standard errors of the mean (SEM). One-way ANOVA, followed by Tukey’s multiple comparisons test was used to analyze data for hot plate, and rotarod tests. All statistical analyses were based on a p < 0.05 level of significance, using GraphPad Prism version 9.0 (GraphPad Software, Inc., San Diego, CA, United States).

**Results**

**Urine sample screening**

Urine samples were analyzed using a 10-panel urine drug test kit. Tested urine samples from Group 1 (Control group) displayed negative results (two clear lines) in all detection zones of the kit, indicating the absence of any illicit drugs or their metabolites in these samples. Whereas, Group 2 (Pregabalin group), and Group 3 (Diazepam group) displayed positive results (one clear line) during the whole period of testing. On the other hand, Group 4 (Microgynon group) displayed negative results (two clear lines) in all detection zones of the kit, which ensures that the kit did not identify Microgynon or its metabolites in urine samples. Moreover, Group 5 (Pregabalin/Microgynon group) displayed positive results despite the administration of OCP, while Group 6 (Diazepam/Microgynon group) displayed uncertain presence nor absence (one clear line and one pale line) of any illicit drugs or their metabolites in urine indicating that more urine tests should be performed.

**Rotarod test outcomes**

Before the real testing, each group received pre-training sessions to ensure the rats became familiarized with the apparatus and the rotating drum. In this test, Group 2, which received Pregabalin for 7 days, and Group 5, which received Pregabalin for 7 days followed by a dose of Microgynon for another 7 days, both exhibited a decrease in the latency to fall. This observed pattern of effect was confirmed by one-way ANOVA, indicating a significant effect of the treatment [F (2, 25) = 5.81, p = 0.0085; Figure 2]. Tukey’s multiple comparisons revealed a significant decrease in latency to fall in Group 2 (Pregabalin group) and Group 5 (Pregabalin/Microgynon group) compared to Group 1 (Control group). In addition, there was a decrease in the latency to fall observed in Group 3, which received Diazepam for 12 days, and Group 6, which was given Diazepam for 12 days followed by a dose of Microgynon for the other 7 days. This observed effect pattern was confirmed by one-way ANOVA, indicating a significant effect of the treatment [F (2, 27) = 33.94, p < 0.0001]. Furthermore, Tukey’s multiple comparisons revealed a significant decrease in latency to fall in both Group 3 (Diazepam group), and Group 6 (Diazepam/Microgynon group) compared to Group 1 (Control group).

**Hot plate test outcomes**

The measurement of rats’ latency to lick their hind paws or jump out of the enclosure of a hot plate apparatus will be used to analyze the effect of illicit drugs and the addition of OCP on their performance.

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Figure 2. Rotarod test outcomes (data are presented as mean ± SEM). (A) Latency to fall in Control, Pregabalin, and Pregabalin/Microgynon groups; (B) Latency to fall in Control, Diazepam, and Diazepam/Microgynon groups (*p < 0.05, ****p < 0.0001; n = 10 for each group).
In this test, Group 2, which received Pregabalin for 7 days, and Group 5, which received Pregabalin for 7 days followed by a dose of Microgynon for another 7 days, showed no difference in the latency to fall between Group 2, and Group 5 compared to Group 1 (Control group). This observed pattern of effect was confirmed by one-way ANOVA, which revealed no significant effect of Treatment \( F(2, 27) = 2.924, p = 0.0709 \); Figure 3. On the other hand, there was an increase in latency time in Group 3, which received Diazepam for 12 days, and Group 6, which received Diazepam for 12 days and then a dose of Microgynon for the other 7 days. This observed effect pattern was confirmed by one-way ANOVA, revealing a significant effect of the treatment \( F(2, 27) = 12.12, p = 0.0002 \). Tukey’s multiple comparison revealed a significant increase in latency time in Group 3 (Diazepam group), and Group 6 (Diazepam/Microgynon group) compared to Group 1 (Control group).

Discussion

The current study explored for the first time the effect of combined oral contraceptives on masking the appearance of abused drugs (Pregabalin and Diazepam) using a 10-panel multi-unit urine screening test. The study’s findings indicate that OCPs have no impact on the detection of illicit drugs in urine, yet, they may lead to invalid findings with certain misused drugs.

Firstly, the current study found that taking pregabalin as well as diazepam resulted in positive urine screening results. During the experiment, one clear line within specific detection zones showed that the kit was able to recognize Pregabalin, Diazepam, and their metabolites in urine.

Moreover, urine tests showed negative results in both the Control and Microgynon groups, indicated by the presence of two clear lines in all the detection zones. This confirms that the urine screening test does not detect anything other than the groups in the kits’ detecting zones (35, 36). In the Pregabalin/Microgynon group, positive results were observed in the urine testing kit, which was confirmed by the appearance of one clear line in the pregabalin detection zones. The addition of OCP could not mask the results of Pregabalin, while the addition of OCP to the Diazepam/Microgynon group showed unclear presence of any illicit drug or their metabolites in urine (one clear line and one pale line). Despite utilizing sufficient urine, proper urine drug test kits, and a cleansed metabolic cage. Previous research has evaluated the specificity and the accuracy of multiple drug rapid detection kits for drug abuse, with results suggesting that these tests can be reliable and appropriate for drug abuse screening in forensic medicine. However, it is important to note that this test produces many false positive results and requires advanced methods to confirm positive results (37). Indeed, this type of test provides preliminary results and cannot be used to detect the concentration of illicit drugs, which should be examined by additional specified methods such as gas chromatography/mass spectrophotometry (38, 39).

Numerous assays were used to assess the impaired motor coordination and the anti-nociception effect of Pregabalin, and Diazepam, both with and without OCP, including rotarod and hot plate tests. The current study’s findings showed that pregabalin, whether administered alone or in combination with OCP, decreased the latency of falls in the rotarod test. However, no change was observed in the latency of expressing pain in the hot plate test. Interestingly, a previous study has shown that Pregabalin administration in a dose of 50 mg/kg did not cause an anti-nociception effect in the hot plate test; though it did result in a 6% reduction in the time spent on the rod (40). In contrast, administering Diazepam alone or with OCP reduced latency to fall in the rotarod test but improved the anti-nociception effect in the hot plate test. A growing body of evidence demonstrates that Diazepam administration in a dose above 1 mg/kg improves the anti-nociception effect, while a dose of 2 mg/kg and above reduces the time spent in the rod (41, 42).

Overall, these results suggest that more frequently abused drugs, such as cannabinoids, cocaine, and marijuana, should be tested with OCPs using gas or liquid chromatography/mass spectroscopy to obtain a better understanding of this phenomenon.

Conclusion

Our research suggests that combined oral contraceptives (OCPs) do not consistently interfere with illicit drug urine testing. However, it appears that OCPs might interact with specific compounds, potentially leading to ambiguous results and providing drug abusers with extended windows before complete drug elimination. Clinically, this underscores the importance of considering the potential influence of OCPs on drug testing outcomes and highlights the need for further investigation into the specific interactions between contraceptives and drug compounds. Future studies are needed to understand the rationale behind the improper use of OCPs to mask illicit drug detection. Serious educational campaigns are also necessary to highlight the negative consequences of OCP intake on men’s health.
References
36. Insert P. REF: 2030612.