INABILITY OF GOLDENSEAL TO INTERFERE WITH THE DETECTION OF MORPHINE IN URINE

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SUMMARY

Preparations of the herb, goldenseal, have been used in an attempt to thwart detection of morphine in urine of human addicts and racing horses. To assess the potential of goldenseal to interfere with equine drug detection, horses were dosed with morphine (0.1 mg/kg IV) and, in one experimental series, also with goldenseal (60 mg/kg, p.o.). Goldenseal resulted in significant increases in urine volume, specific gravity and acidity. A significant decrease in total morphine excreted occurred 5-6 hours after dosing. It was concluded that with inclusion of an efficient hydrolysis step in the analysis, goldenseal was unlikely to interfere with urine tests for morphine, even if water loading was included in the dosing regimen.

INTRODUCTION

Goldenseal (*Hydrastis canadensis*) was called "the herb of the Cherokees" and was widely used by American settlers as a herbal medicament for many ailments ranging from eye inflammation to a dye.[12] Although modern medicines have largely replaced goldenseal, it is still used in some home remedies. In the United States, goldenseal is included in one over-the-counter preparation[16] and in 36 compounds available over the counter on the Canadian market.[6] The literature indicates greater widespread use in Europe and the Far East.

As an herbal tea, goldenseal has been most commonly used as a general tonic.[7,10,11,12,13] More specific uses have included treatment of uterine hemorrhage,[6] cleansing the eye, improvement of appetite, healing the liver, treatment of a sore throat[10] and use as a laxative.[6] In recent years, drug testing laboratories have received sporadic reports of a new use of this compound. Reports and rumors have suggested goldenseal could be used to avoid detection of morphine in urine samples from humans and horses who have received either morphine or heroin.

Goldenseal contains three major alkaloids: hydrastine, berberine and canadine, as well as volatile oils and a resin.[9] Berberine has been found in horses by racing chemists in the United States and Canada,[15,17] suggesting that goldenseal has been available to racing horses. The present study was undertaken to evaluate the ability of goldenseal to interfere with the detection of morphine in equine urine.

MATERIALS AND METHODS

Following initial draining of the urinary bladder by catheterization, four Thoroughbred and Standardbred mares, weighing between 410 and 512 kg, were dosed intravenously (IV) with 0.1 mg morphine[a] per kg body weight. Blood samples were drawn 1 and 2 hours later and every other hour thereafter for 12 hours. The urinary bladder was emptied by catheterization at hourly intervals for 12 hours. The total volume of each specimen was recorded, as was the pH and specific gravity. Urine and serum samples were stored at -20° C until the morphine analysis was performed. Throughout the 12-hour duration of the experiment, the horses were allowed hay and water *ad libitum*. One month later the experiment was repeated on the same four horses except that immediately preceding the administration of morphine, the horses were orally dosed with 60 mg

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goldenseal[b] per kg body weight.

The analytical method for determination of morphine levels in equine serum and urine has been reported previously.[3] Urine samples hydrolyzed were incubated for 4 days at 37° C with an equal volume of Glucurase®,[c] a β-glucuronidase preparation used to free morphine from its glucuronide complex. Sample purification was accomplished by liquid-liquid extraction and column chromatography. The morphine derivative was quantitated by gas chromatography.

Serum morphine levels following administration of 0.1 mg/kg morphine IV alone and the same amount of morphine plus 60 mg/kg goldenseal are shown in Figure 1. Initially, serum morphine levels were slightly higher when goldenseal was included in the dosing regimen, and then slightly lower. However, there was no statistically significant difference in serum levels following the 2 treatments (paired data t-test, t = .96, P < .05).

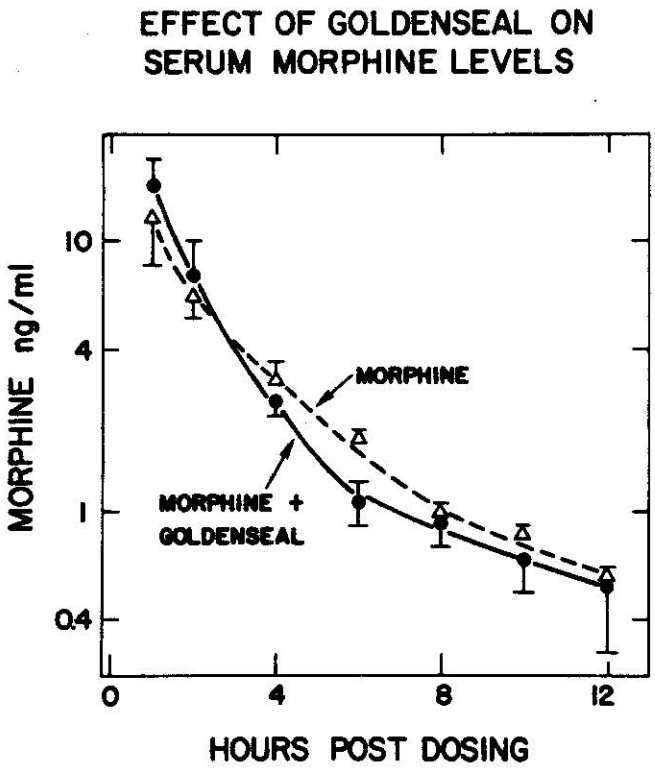

Figure 1. Morphine serum levels were determined on four horses that had been dosed IV with 0.1 mg/kg morphine (shown by the open triangles △) and the same amount of morphine plus 60 mg/kg goldenseal p.o. (shown by the closed circles •-•). Vertical bars represent SEMs.

In the presence of goldenseal, morphine levels in unhydrolyzed urine samples from the same horses (Figure 2) were slightly lower by 6 hours post dosing, but this difference was not statistically significant (paired data t-test, t = 1.33, P < .05).

[b]Humco Laboratory, Texarkana, TX.
[c]Sigma Chemical Co, St. Louis, MO.

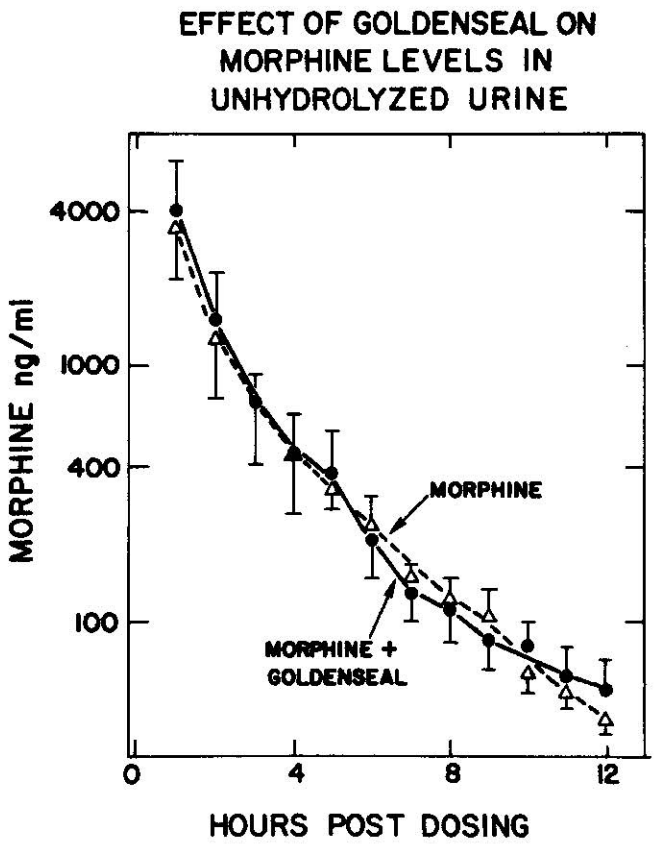

Figure 2. Morphine levels were determined on unhydrolyzed samples from four horses following IV dosing with 0.1 mg/kg morphine (open triangles △) and the same amount of morphine plus oral administration of 60 mg/kg goldenseal (closed circles •-•). SEMs are represented by the vertical bars.

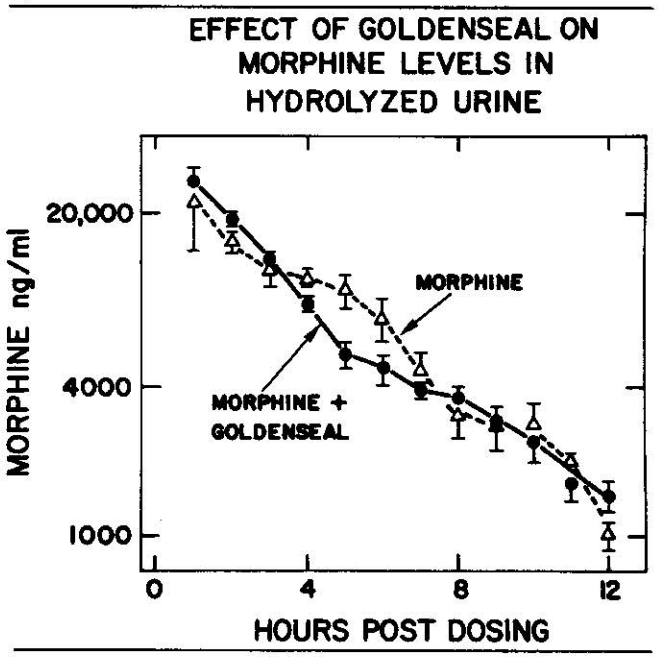

Figure 3. Morphine levels were determined on hydrolyzed urine samples from four horses following IV dosing with 0.1 mg/kg morphine (open triangles △) and the same amount of morphine plus oral adminstration 60 mg/kg goldenseal (closed circles •-•). SEMs are represented by the vertical bars.

Morphine levels in urine samples which had been hydrolyzed (Figure 3) showed similar patterns. Urine morphine levels were initially higher in the goldenseal treated horses but dropped below control morphine levels by 4 hours before returning to above or equivalent to control levels by 7 hours. In this case, a paired data t-test indicated a significant difference between the 2 treatments at 4-6 hours after dosing ($t = 5.46$, $P = .05$). However, when data points from all 12 hours were included, there was no significant difference (paired data t-test, $t = .015$, $P < .05$).

The number of µg of morphine recovered in each 1-hour period was calculated for both the morphine and the morphine plus goldenseal treatment. The increase in morphine levels following hydrolysis over free morphine levels found in unhydrolyzed urine samples was plotted in Figure 4. Immediately following dosing, the inclusion of goldenseal in the treatment appeared to result in a larger percentage of the drug being excreted in the water soluble glucuronide form. Although the SEMs do not overlap at 1 hour, these differences were not statistically significant when all points were compared in a paired data t-test ($t = .01$, $P < .05$).

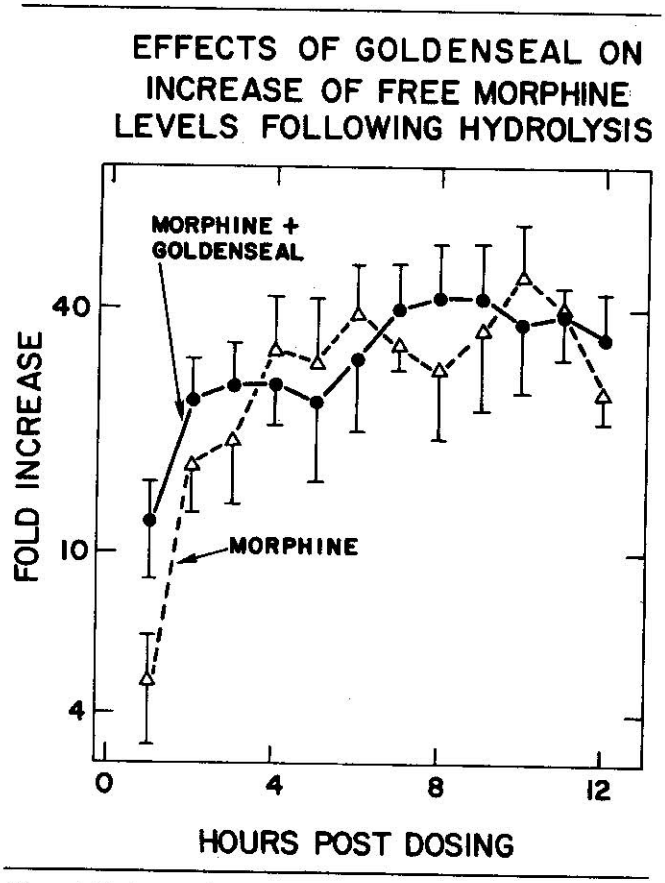

Figure 4. The increase in morphine levels following hydrolysis over free morphine levels found in unhydrolyzed urine samples was determined following dosing with 0.1 mg/kg morphine (open triangles △) and morphine plus 60 mg/kg goldenseal (closed circles •-•). SEMs are represented by the vertical bars.

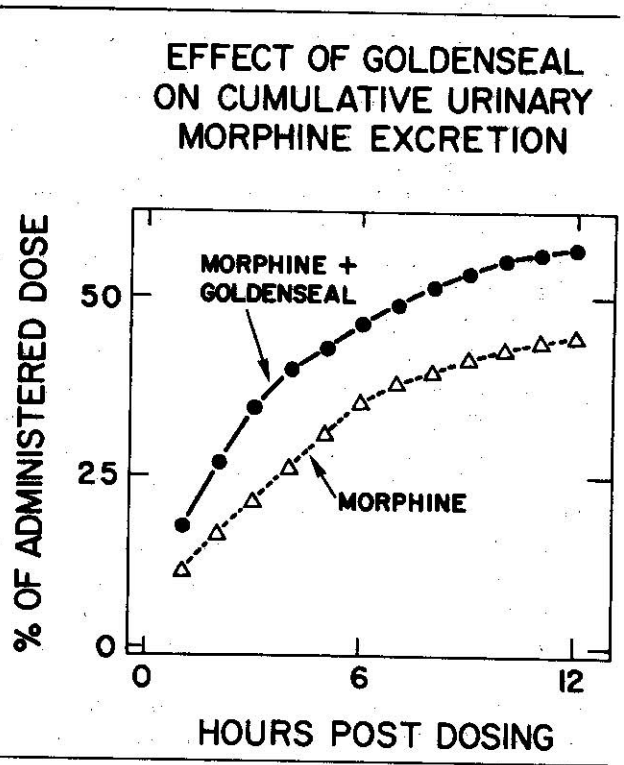

Figure 5. Four horses were dosed with 0.1 mg/kg morphine and with morphine plus 60 mg/kg goldenseal. The cumulative percentage of the administered drug recovered from the urine is shown by the (open triangles △) following just morphine administration and by the (closed circles •-•) following treatment with both morphine and goldenseal.

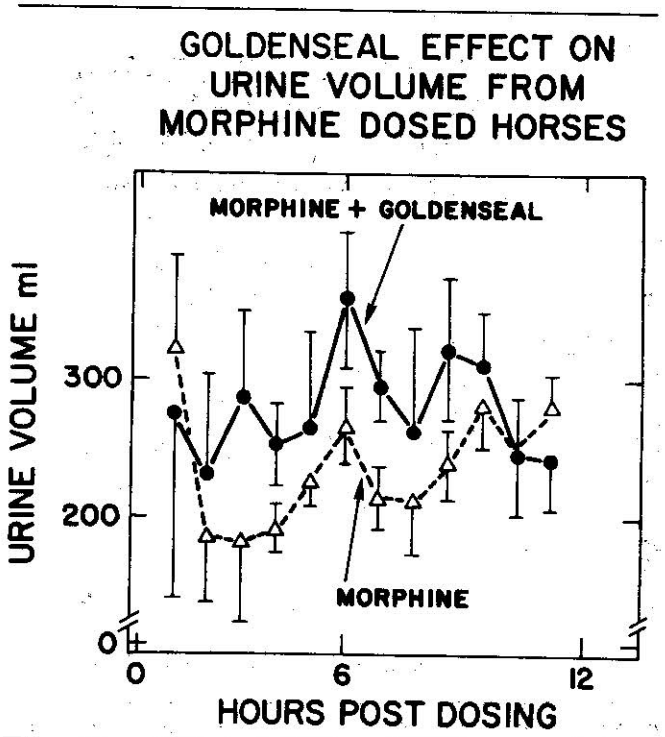

Figure 6. Urine volume was determined hourly from horses dosed with 0.1 mg/kg morphine (△) and from horses given both morphine and 60mg/kg goldenseal (•-•). SEMs are represented by the vertical bars.

Although statistically significant differences were not found on the basis of an hour by hour comparison, Figure 5 shows that when the cumulative percentage of administered drug recovered from the urine is compared for treatments with and without goldenseal, there is a statistically significant increase (paired data t-test, $t = 19.26$, $P < .05$) following administration of goldenseal.

Urine volume was increased in the presence of goldenseal (paired data t-test, $t = 2.90$, $P < .05$) as shown by Figure 6. This suggested the possibility that although the ng morphine per ml urine were similar with and without goldenseal, the increase in urine volume might be sufficient to make a significant difference in the total amount of morphine excreted each hour. Althouh the t-value in the paired data t-test was increased from .015 to 1.63, this still was not statistically significant.

Despite the volume increase in the presence of goldenseal, there was an accompanying small but significant increase in urine specific gravity when goldenseal had been administered (paired data t-test, $t = 2.42$, $P = .05$) which was particularly noticeable during the first 4 hours (Figure 7).

Urinary pH was consistently lower between 3 and 10 hours after dosing when goldenseal was included. Even when values for all 12 hours were evaluated in a paired data t-test, there was a statistically significant difference ($t = 3.08$, $P < .05$).

DISCUSSION

The alkaloids of goldenseal have been isolated and studied individually for specific physiological effects. Hydrastine constricts peripheral vessels which has lead to its use in the treatment of postpartum hemorrhage and nosebleeds.[10] Berberine has an acetylcholine potentiating effect which has been shown to cause a marked but transient hypotension in rats, rabbits, cats and dogs.[5] It has also been found to have antibacterial, antimalarial and antipyretic activity[4] which account for its usefulness in treatment of sore eyes, general ulceration and perhaps for liver disorders and as a tonic.[7,10,11,13] Canadine is a sedative and muscle relaxant.[14]

None of the known actions of any of the alkaloids found in goldenseal, nor any of the home remedies suggest any way in which goldenseal could prevent the detection of morphine in a subject's urine. Herbal medicine recipes and directions related by heroin addicts trying to "beat" the drug detection test always involve the ingestion of large quantities of fluid, the herb most often being prepared as a tea. Gorodetzky,[8] using human volunteers, did a series of experiments involving the administration of heroin, goldenseal and 72 ounces of water, alone and in various concurrent combinations. He concluded that goldenseal, without water, did not change the time course of detection of morphine, indicating that the herb was not affecting the metabolism of heroin. Water loading did result in more dilute urine in the first 8-hour period but did not reduce the effectiveness of his test. Presumably, this was because morphine levels were so high during this period that the dilution did not bring

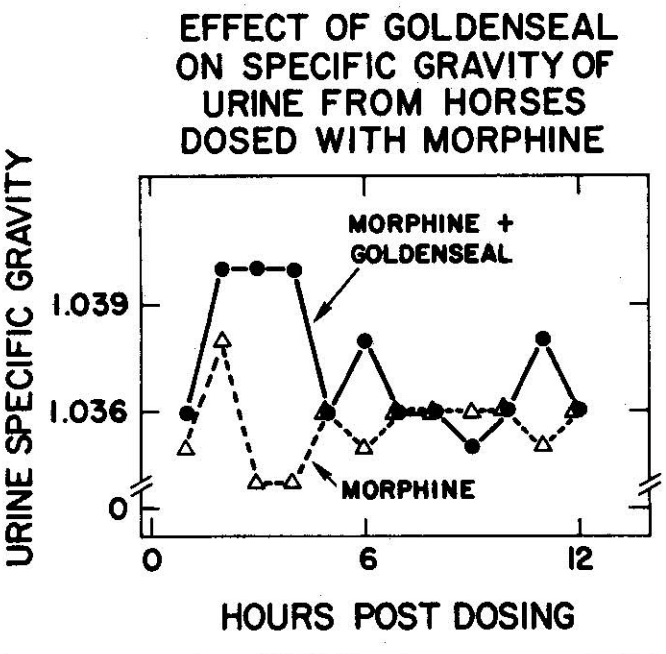

Figure 7. Urine specific gravity was checked at hourly intervals on samples from horses dosed with morphine (shown by open triangles △) and those dosed with both morphine and goldenseal (shown by the closed circles •-•).

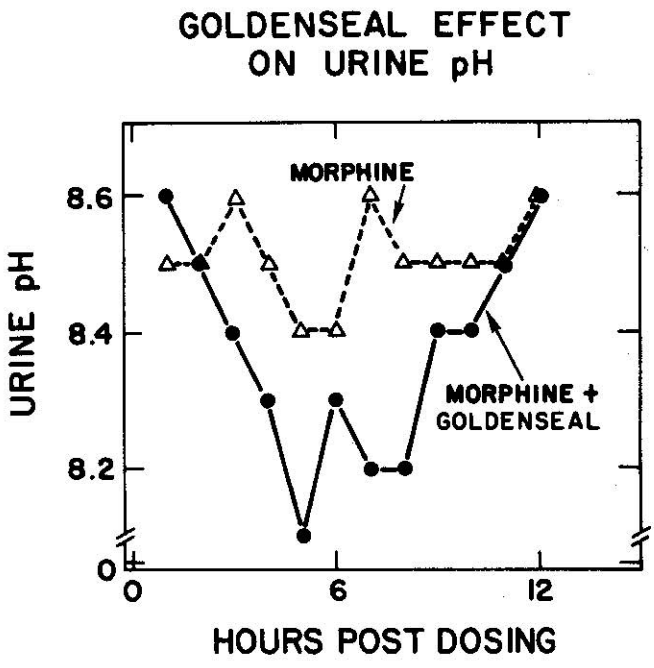

Figure 8. Urinary pH was determined on samples taken at hourly intervals from horses given morphine (shown by open triangles △) and those given morphine plus goldenseal (shown by the closed circles •-•).

the levels below the limit of sensitivity of the assay being used, generally in the range of 100 ng/ml. Gorodetzky did find, however, that water plus goldenseal resulted in dilute urines only in the second 8-hour period following drug administration. In this case, the goldenseal appeared to have delayed the peak of diuresis until the falling morphine levels were already low; thus, further dilution at this time reduced the concentration of morphine below the threshold of his test's sensitivity.[8]

Water loading was not included in the experimental design of this work done with horses, because it was expected that any effect of goldenseal itself would be more easily isolated. The serum morphine concentrations, as well as levels in both hydrolyzed and unhydrolyzed urine, indicated that goldenseal alone had a smaller effect on morphine levels than if large quantities of fluids had also been consumed. However, goldenseal did have a consistent, maximal effect 4-6 hours after p.o. administration. In contrast to Gorodetzky's conclusion that metabolism was not affected, this data, most clearly seen in Figure 5, indicates that a small but significant increase ($P<.05$) in the drug excreted in the urine is in the form of a glucuronide complex.

The simultaneous increase in urine volume and specific gravity (Figures 6 and 7) seen in the presence of goldenseal, indicates that the normally hydrated horse actually lost fluids and solids not normally excreted. Although the possibility exists that goldenseal is a diuretic (this is doubtful in view of Gorodetzky's work), it must be remembered that morphine is an antidiuretic. Perhaps the goldenseal is either blocking or counterbalancing this action of morphine.

Along with the fact that more morphine was in the water soluble glucuronide form and that a larger volume of fluid was being eliminated, another contributing factor to the morphine excretion pattern in the presence of goldenseal was the increase in urine acidity. This would particularly facilitate the excretion of free morphine.

The maximum increase in glucuronide production was at 1-3 hours, the largest volume difference was at 3 hours, the largest increase in specific gravity was at 3-4 hours, the highest acidity was not seen until 5 hours and the maximum decrease in total morphine levels was observed between 5 and 6 hours. A possible explanation for this time course of events lies in the fact that of the 2 major alkaloids found in goldenseal, hydrastine is insoluble in water, while berberine is water soluble,[1] making it likely that they were absorbed at different rates from the stomach. Hydrastine and berberine, as well as alkaloids appearing in smaller quantities, were likely primarily responsible for different effects. The overall result of the ingestion of goldenseal was for an initially larger amount of morphine to be excreted, due mainly to the increased glucuronide formation. By the time there was an increased urine volume, the morphine load had been lowered and the morphine remaining was being excreted in a larger volume, resulting in lower morphine levels per ml urine in the presence of goldenseal. Many morphine assays have 100 ng/ml as the lower limit of detection. Although it is possible that the goldenseal effects will be sufficient to decrease morphine levels in unhydrolyzed urine to below this cut-off value, without the added diluting effect resulting from water loading, it is unlikely that this significant but relatively small drop in urine morphine levels would result in the drug escaping detection at all.

The use of an efficient β-glucuronide hydrolysis[2] would make it even less likely that goldenseal could be effective in interfering with the urine test for morphine. Despite the fact that morphine levels in unhydrolyzed urine specimens from goldenseal-treated horses had fallen below 100 ng/ml by 9 hours post dosing, with hydrolysis all urine samples contained over 1000 ng morphine per ml through 12 hours. In fact, from other work we have reported[3] hydrolyzed urine samples from horses receiving this same dose of morphine did not fall below 100 ng/ml until 48 hours after drug administration.

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