



Influence of Co-Administration of Bioflavonoid on Diuretic Activity of Furosemide

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

As bioflavonoids have a strong affinity to bind with albumin, it is plausible that they may have the ability to displace the diuretic furosemide bound to albumin. In this study we sought to verify this hypothesis by examining the effect of the co-administration of a bioflavonoid with furosemide on the diuretic activity of the latter. Diosmin is a bioflavonoids type of plant chemical found mainly in citrus fruits.

For this purpose, we analyzed bioflavonoids by their ability to bind to human serum albumin (HAS) using an *in silico* method and found that diosmin had a higher affinity to albumin than furosemide. Subsequently, we investigated the effect of the co-administration of diosmin with furosemide on the diuretic activity of the latter in mice.

Our results showed that the combination did not produce any significant change in the diuretic activity of furosemide; however, after 3 hours of treatment, the urine volume of the mice that received diosmin along with frusemide was greater than that of mice administered only the same dose of furosemide. There was no significant difference in urine volume between the two groups at the end of 24 hours. A similar trend of increased levels at 3 hours in the combination group and absence of any difference at 24 hours was noted in the case of the urine concentrations of Na⁺, K⁺, and 2 Cl.

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Our findings indicate that co-administration of diosmin increased the immediate diuretic effect of furosemide for the first few hours and that this effect subsides within 24 hrs. Therefore, this combination should be used with care, especially during the first few hours of administration.

Keywords: Bioflavonoids; diosmin; furosemide; urine output; diuretic.

1. INTRODUCTION

Drug interactions with albumin are known to have both detrimental and beneficial effects [1,2]. The anthranilic acid derivative furosemide is a widely used diuretic drug. Furosemide, a loop diuretic, primarily acts by inhibiting the luminal $\text{Na}^+/\text{K}^+/2\text{Cl}$ co-transporter systems in the thick ascending limb of the loop of Henle [3]. The percentage of furosemide binding to the plasma albumin is 99% [4]. This strong affinity of furosemide to bind with albumin can lead to high serum concentrations of the drug, resulting in serious consequences.

Another molecular group that shows a strong affinity to bind to plasma albumin is the flavonoid group. Flavonoids are natural polyphenols that have performing a scavenging function and other important physiological effects such as strong plasma protein binding with competing ability [5,6] or inhibition of different enzymes and transporters [7]. Numerous studies, both *in vivo* and *in vitro*, have shown that flavonoids are powerful antioxidants effective against a wide range of diseases including cancers, allergies, and different free-radical-mediated disorders, e.g., atherosclerosis, ischemia, neuronal degeneration, and cardiovascular disorders [8]. Diosmin is a bioflavonoids type of plant chemical found mainly in citrus fruits.

The concurrent administration of flavonoids with furosemide may cause displacement of the furosemide from plasma albumin. Hence, there is a high possibility for drug–drug interaction with change in furosemide activity, which would not be desirable.

This study was designed to compare the diuretic effect of furosemide when administered alone and in the combination with a bio-flavonoid (diosmin) in Swiss albino mice.

2. MATERIALS AND METHODS

2.1 Study Design

This comparison of the diuretic activity of furosemide administered alone or in combination with a bioflavonoid (diosmin) was performed on

Swiss albino mice. Initially a suitable dose of furosemide was selected, followed by the selection of a suitable diosmin.

Suitable bioflavonoids with an affinity for albumin were evaluated. One bioflavonoid (diosmin) was selected and tested for its effect on the diuretic activity when administered along with furosemide. The study protocol was approved by the institutional review board (PH8/201).

2.2 Selection of Bioflavonoids

Among the several bioflavonoids, the most suitable one for this study was identified on the basis of data regarding affinity to bind with human serum albumin (HSA). The *in silico* method for drug design was used for this purpose. The data on the binding affinity were analyzed with UCSF Chimera software (the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco). This software was utilized for its capability in providing an interactive visualization and analysis of molecular structures and related data.

2.3 Dose Selection of Furosemide and Diosmin

Furosemide was obtained from the local pharmacy (Lasix®; Sanofi, France). Furosemide and Diosmin (Molecular weight: 608, purity 99%, Fluka Chemica, Busch, Switzerland) were dissolved in water and administered orally to the mice. The dose was calculated based on previous experiments conducted on Swiss albino mice and as described by formula for determining the human equivalent dose (HED) for administration in animals [9]:

$$\text{Animal Dose (mg/kg)} = \text{HED (mg/kg)} \times [\text{Human Km} / \text{Animal Km}]$$

where

$$\text{Human Km} = 37; \text{ Mouse Km} = 3$$

Accordingly, the required dose of furosemide and diosmin were determined to be 7.05 mg/kg and 102.7 mg/kg respectively for Swiss albino mice.

2.4 Animals

Swiss albino mice (both sexes; weight, 25–30 g; N = 30) were used. All animals were housed in metabolic cages with *ad libitum* access to food and water. Urine samples were collected using metabolic cages, and the samples were analyzed for volume and concentrations of Na⁺, K⁺, and 2Cl by using a previously described method [10].

2.5 Study Groups

The animals were divided into five equal groups (n=6 mice, each). Group 1 (control group), served as a control group treated with vehicle. Group 2 (FUR-I), low dose furosemide treated group (7.05 mg/kg). group 3 (FUR-II), high dose furosemide treated group (14.09 mg/kg). Group 4, diosmin treated group (102.75 mg/kg). Group 5 (FUR-I+diosmin), received a combination of furosemide (7.05 mg/kg) and diosmin (102.75 mg/kg).

After oral administration of the appropriate drug(s) as a single dose for each group, the diuretic activity was assessed. The urine volume was measured at 3 and 24 hours (h) to determine immediate and late effect. In addition, the concentrations of the Na⁺, K⁺, 2Cl in urine were also assessed at these time points. The results were expressed as mean±standard error of the mean (SEM).

3. RESULTS

3.1 Selection of Bioflavonoid

In silico analysis of the data of the binding affinity of bioflavonoids to HSA revealed that diosmin was a suitable candidate due to its high affinity to HAS (Table 1).

3.2 Diuretic Activity

Diuretic activity was assessed in terms of the urine volume. Fig. 1 shows the urine volumes at 3 h and 24 h in the five study groups. The urine volume in the diosmin group was similar to that in the control group, which indicated that diosmin

does not have diuretic activity at the dose administered in this study. On the other hand, the urine volume in the FUR-II group was higher than that in the FUR-I group, thereby confirming that furosemide causes a dose-dependent increase in urine volume in our study animals; moreover, this difference was noted at both 3 h and 24 h, which implies that the dose-dependent increase in urine volume induced by furosemide was maintained throughout the 24-h assessment period.

Further, the urine volume at 3 h was the highest in the FUR-II group and lowest in the Diosmin group (Fig. 1). A similar trend in urine volume was noted at 24 h.

Interestingly, the urine volume in the FUR-I+Diosmin group was greater than that administered in the FUR-I group in at 3 h, but this difference was negligible at 24 h (Fig. 1). The difference between the two groups at 3 h was not statistically significant.

3.3 Ion Concentrations

The urine concentrations of Na⁺, K⁺, and 2Cl were measured at different time intervals. The concentrations of the ions at 3 h were higher in the FUR-I+Diosmin group than in the FUR-I group; however, there was no difference at 24 h (Figs. 2-4).

4. DISCUSSION

This study was comprised of two substudies. The first substudy, involved *in silico* analysis to identify a suitable bioflavonoid with a high affinity for HSA. Analysis indicated that diosmin was a suitable candidate. The second substudy involved evaluating the effect of administering diosmin with furosemide on the diuretic activity of the latter. The results of the current study indicated that the co-administration of a bioflavonoid with furosemide led to a temporary increase in the urine volume and that this effect subsided within 24 hours. Along with an increase in urine volume, diosmin also temporarily enhanced the urine concentrations of Na⁺, K⁺, and 2Cl.

Table 1. Binding affinities of the bioactive constituents towards human serum albumin (HSA)

S. No.	Active constituents/ Enzymes	Binding affinity (kcal/mol)
1.	Naringenin	-8.5
2.	Diosmin	-10.2
3.	Quercetin	-8.3
4.	Furosemide	-8.0
5.	Cocrystallized ligand (camptothecin)	-8.2

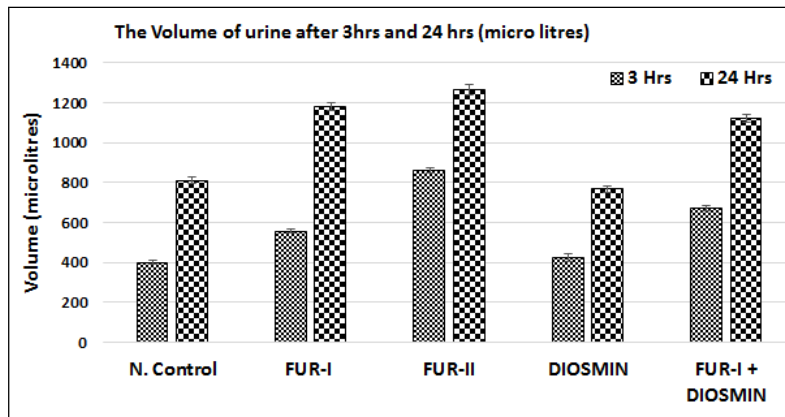


Fig. 1. Volume of urine in the five groups at 3 hours and 24 hours
 The doses of furosemide used were 7.05 mg/kg (FUR-I) and 14.09 mg/kg (FUR-II) and the dose of diosmin was 102.75 mg/kg

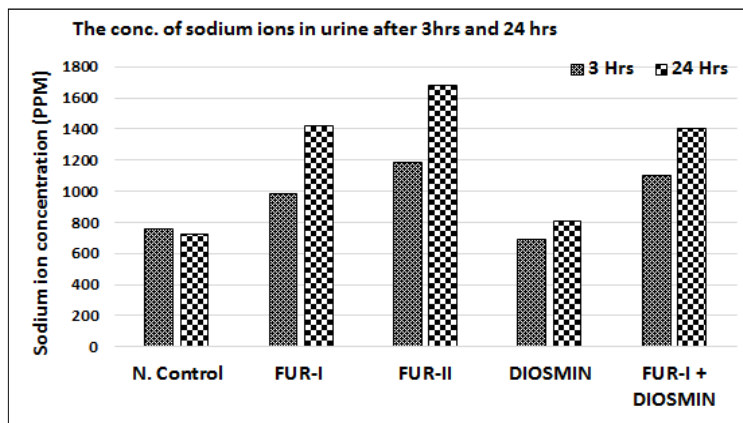


Fig. 2. Sodium ion concentrations in urine at 3 hours and 24 hours
 The doses of furosemide used were 7.05 mg/kg (FUR-I) and 14.09 mg/kg (FUR-II) and the dose of diosmin was 102.75 mg/kg

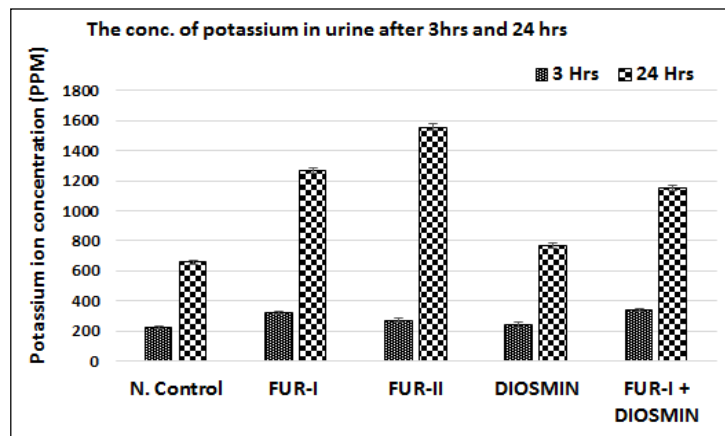


Fig. 3. Potassium ion concentrations in urine at 3 hours and 24 hours
 The doses of furosemide used were 7.05 mg/kg (FUR-I) and 14.09 mg/kg (FUR-II) and the dose of diosmin was 102.75 mg/kg

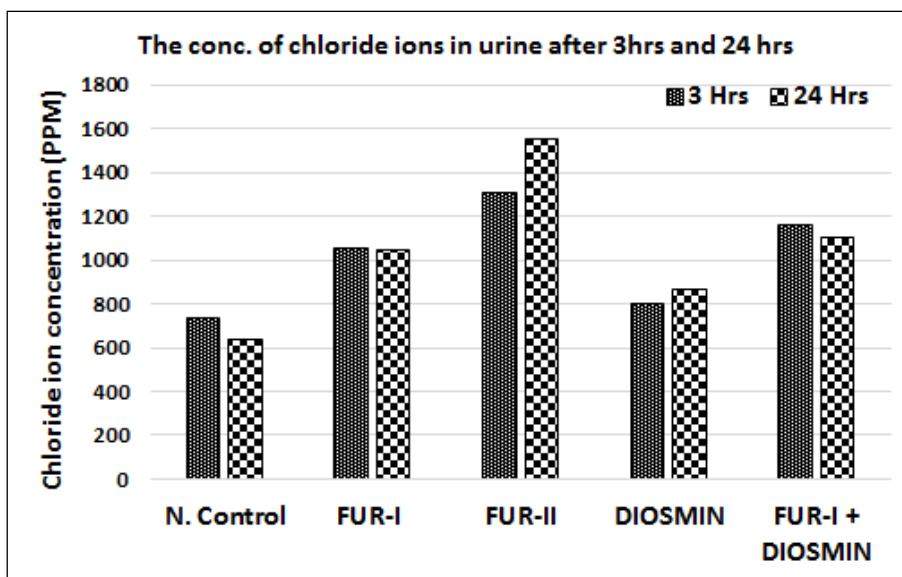


Fig. 4. Chloride ion concentrations in urine at 3 hours and 24 hours

Bioflavonoids have been known for their unique properties and have attracted a considerable attention in recent years, especially with regard to their binding affinity to albumin [11]. This property of the flavonoids has been examined in several studies and has implications in several conditions [12,13]. For example, patients taking drugs which are P-glycoprotein substrates may have to restrict their intake of bioflavonoid-containing foods and beverages [13,14]. The bioflavonoid diosmin, has been evaluated for its renoprotective and hepatoprotective effects [15, 16]. The initial substudy identified diosmin as a suitable candidate for the subsequent substudy because it has a higher affinity to HSA as compared to the diuretic drug furosemide. Both furosemide and diosmin bind HSA at site I, indicating that both compete for binding HSA [17,18]

In the current study, diosmin at the administered dose did not exert have any diuretic effect as indicated by the lack of increase in urine volume in the Diosmin group. However, when the diosmin was administered along with furosemide (FUR I + Diosmin group), there was an increase in urine volume compared to that observed in the FUR-I group. This implies that the improved diuresis observed in the FUR-I + Diosmin group was due to potentiating the action of furosemide rather than an additive effect. A similar effect has been reported between diosmin and interferon-alpha [19]. Further investigations are necessary to determine whether the lack of a

statistically significant differences between the FUR-I and FUR-II groups -and the FUR-I+Diosmin group was due to the small sample size in this study.

Furthermore, the increase in the urine volume was observed at 3 h, but not at 24 h, indicated that the diuresis-enhancing effect of diosmin was temporary. This could be attributed to the alterations in the plasma concentration of drug occurring due to changes in both the clearance of the drug and its volume of distribution [20]. The exact nature of the interaction between diosmin and furosemide requires further studies.

There are some limitations to this study. First is the small sample size, it is possible that the lack of significance in the intergroup difference in urine volume could be due to the small sample size. Second, only a single dose of diosmin was examined in this study. Additionally, measurement of renal clearance of furosemide could also provide further insight into the effect of diosmin on the diuretic activity of furosemide.

5. CONCLUSION

The results of this study indicate that combination of furosemide with diosmin temporarily enhances the diuretic activity of furosemide by increasing both urine output and clearance of Na^+ , K^+ , and 2Cl^- . This implies that co-administration of diosmin may enhance the diuretic activity of furosemide without the need

for increase the dose of the furosemide. Additionally, caution is urged when this combination is used, particularly in the early hours after administration.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments were conducted in accordance with the National Health guidelines for the welfare of experimental animals and with the approval of the Ethical Committee.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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