

Effect of *Murraya koenigii* pretreatment on the transport of buspirone across rat intestine

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Abstract

To investigate the effect of *Murraya koenigii* fruit (aqueous extract) pretreatment on transport of buspirone, a CYP3A4 substrate across the rat intestine. The transport of buspirone across rat intestine (duodenum, jejunum and ileum) was studied by using non-everted sac method. The rats were pretreated with *Murraya koenigii* fruit (aqueous extract) for 7 days. The rats were sacrificed by using anesthetic ether, the intestinal segments were isolated and used for the studies. The probe drug (buspirone) solution was placed in the isolated intestinal sac. Samples were collected at preset time points and replaced with fresh buffer. The drug content in the samples was estimated using high performance liquid chromatography method. Control experiments were also performed. The results revealed that there was a significant ($p < 0.05$) difference compared to control, in the transport of buspirone from the intestinal sacs which were pretreated with *M. koenigii* fruit (aqueous extract). It suggests that both *M. koenigii* aqueous extract might be acting by inhibiting the transporters and enzymes which are responsible for transport/metabolism of buspirone. From the results it can be concluded that *M. koenigii* fruit aqueous extract might be acting by inhibiting CYP3A4 enzymes as buspirone is extensively metabolized by CYP3A4. Further studies are recommended to prove their effects in human beings.

Keywords: Cytochrome P-450 3A, noneverted sac, *Murraya koenigii*

Introduction

Natural products, as used by the general population, are usually complex mixtures of many compounds. Both the putative active ingredient(s) and other constituents present in that mixture have the potential to cause interactions with various classes of drugs. Such interactions include induction or inhibition of metabolizing enzymes and drug efflux proteins. Prokaryotes and eukaryotes cannot distinguish between a natural chemicals originating from plants and a chemical synthesized in laboratory. Consequently, physiological, pharmacological and toxicological effects of these chemicals irrespective of their origins remain the same. Ever-increasing use of herbs with western medicines raises the potential for drug-herbal interactions,

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which may alter drug bioavailability through altered absorption, distribution and metabolism. Primary mechanisms of drug/herb interaction involve either induction or inhibition of intestinal drug efflux pumps [efflux proteins such as P-glycoprotein (P-gp) and multiple resistance proteins (MRPs)] and intestinal and hepatic metabolism by cytochrome P450s (CYPs) (Evans 2000, Ioannides 2002). A concerted action by both drug efflux pumps and CYPs lower oral bioavailability of many drugs, i.e., protease inhibitors, macrolides and azoles. The most versatile enzyme system involved in the metabolism of xenobiotics is cytochrome P450. The CYP3A family of enzymes constitutes the most predominant phase-I drug metabolizing enzymes and accounts for approximately 30 % of hepatic CYP and more than 70 % of intestinal CYP activity. Moreover, CYP3A is estimated to metabolize between 50 % and 70 % of currently administered drugs (Watkins 1997). A congener of CYP family is CYP3A4, the most abundant form (Kolars et al. 1992). This CYP3A4 enzyme is present primarily in the hepatocytes and enterocytes (Parkinson 1996). It is now fairly established that naturally occurring dietary supplements can modulate hepatic and enterocytic CYP activity. Perhaps the best documented clinically relevant drug interaction is observed with grape fruit juice. Simultaneous consumption of grape fruit juice with a number of therapeutic agents that are subject to first pass intestinal/hepatic metabolism, resulted in higher plasma levels with subsequent adverse effects (Bailey et al., 1998). Grape fruit juice acts through inhibition of intestinal CYP3A4, which regulate pre-systemic metabolism (Guo et al. 2000). Although hepatic biotransformation can make a major contribution to systemic drug elimination, a combination of hepatic and intestinal drug metabolism may cause significant pre-systemic or first-pass drug loss. Use of herbal drugs has been increased enormously because of their efficacy coupled with decreased risk of side effects.

Murraya koenigii fruit pulp contains 64.9 per cent moisture. The content of total soluble solids of the aqueous extract is 16.8 per cent. The pulp contains 9.76 per cent total sugars, 9.58 per cent reducing sugars, 0.17 percent non-reducing sugars and almost a small amount of tannins. The mineral content of the edible portion of the fruit, as represented by its ash, is 2.162 per cent. Similarly, 100 g of the edible portion of the fruit contains protein, 1.97 g; phosphorus, 0.082 g, potassium, 0.811 g, calcium, 0.166 g; magnesium, 0.216 g; and iron, 0.007 g. Strong odiferous oil occurs in the leaves and the seeds of *M. koenigii* (L.) Spreng. The chemical examination of this oil reported that this essential oil exhibited a strong antibacterial and antifungal activity. The shiny-black fruits are liked both by children and adults. As revealed by the chemical composition of the fruits, they are very nutritious. These fruits have also many medicinal properties (Mallavarapu et al. 2000).

Materials and Methods

Buspirone hydrochloride was gifted by Sun Pharmaceuticals, Dulbecco's Phosphate Buffer pH 7.4, Hi Media Ltd., India, Methanol HPLC grade, Merck Ltd., India, Acetonitrile HPLC grade, Merck Ltd., India, were used. All other chemicals used were of analytical grade.

Preparation of M. koenigii aqueous extract

M. koenigii fruits were collected and washed. The seeds were grinded in mixer (Remi, India). The freshly prepared aqueous extract was administered to rats. The fruits used for the study were collected from same plant.

The animal study was conducted according to the protocol approved by animal ethics committee, Kakatiya University, India. Male wistar rats weighing 200 ± 25 g were selected for experiments. *M. koenigii* aqueous extract was administered to rats ($n=3$) at a dose of 10 mL kg^{-1} for seven days. Untreated rats ($n=3$) were used as control.

The rats were fasted overnight with free access to water before the experiments. Control rats and pretreated rats on seventh day were sacrificed using anesthetic ether, the intestine was surgically removed and flushed with 50 mL of ice cold saline. The small intestine was cut into 3 segments, duodenum, jejunum and ileum of equal length (10 cm). The probe drug (Buspirone 10 mg mL^{-1}) was dissolved in pH 7.4 isotonic Dulbecco's PBS (D-PBS) containing 25 mM glucose. The probe drug solution (1 mL) was filled in the normal sac (mucosal side), and both ends of the sac were ligated tightly. The sac containing probe drug solution was immersed in 40 mL of D-PBS, containing 25 mM glucose in the mucosal side. The medium was pre-warmed at 37°C and pre-oxygenated with 5 % CO_2 / 95 % O_2 for 15 min, under bubbling with a CO_2/O_2 mixture gas, the transport of the buspirone from mucosal to serosal surfaces across the intestine was measured by sampling the serosal medium periodically for 120 min. The samples of 1 mL were collected at predetermined time intervals from the serosal medium and replenished with fresh buffer. The drug transported was measured using high performance liquid chromatography (HPLC) method.

HPLC analysis

Shimadzu HPLC system equipped with a LC-10AT pump and SPD 10 AT UV visible detector and RP C18 column (250 mm x 4.6 mm ID, particle size 5μ) was used for the analysis of samples. The mobile phase used was a mixture of (30:70) of acetonitrile, buffer (0.02M of NaH_2PO_4 , pH 2.5). The flow rate was 1 mL per min and the detection was carried at 240 nm. A calibration curve was plotted for buspirone in the range of $1\text{--}40 \mu\text{g mL}^{-1}$. A good linear relationship was observed between the concentration of buspirone and the peak area of buspirone with a correlation coefficient ($r^2 = 0.999$). The required studies were carried out to estimate the precision and accuracy of the HPLC method of analysis of buspirone.

Sample preparation

To 200 μL of intestinal sac samples, 100 μL of methanol was added and vortexed on a cyclo-mixer for two min and centrifuged at 5000 rpm for 15 min using Biofuge Fresco Centrifuge (Heraeus, Germany). The supernatant (20 μL) was injected into HPLC.

Statistical analysis

The results were tested for statistical significance using t-test. The difference in the sample means were considered significant at $p < 0.05$.

Results and Discussion

In the present study, the mean cumulative transport of buspirone from mucosal to serosal (normal sac) was determined in duodenum, jejunum and ileum regions of rat intestine in the absence and presence of *Murraya koenigii* aqueous extract pretreatment. The time course of buspirone transport at different concentrations across rat small intestine of duodenum, jejunum and ileum was shown in Table 1.

Table 1. Mean \pm S.D (n=3) cumulative transport ($\mu\text{g/mL}$) of buspirone (10 mg mL^{-1}) in intestinal sacs in Wistar rats

Region	Control	Buspirone + <i>M. koenigii</i> (1mL)	Buspirone + <i>M. koenigii</i> (2mL)
Duodenum	55.87 \pm 8.81	1098.89 \pm 19.60	4682.67 \pm 54.24
Jejunum	137.86 \pm 16.10	1165.54 \pm 83.99	5720.43 \pm 60.23
Ileum	165.23 \pm 7.87	712.01 \pm 43.13	3319.33 \pm 52.80
Colon	176.70 \pm 13.76	1241.13 \pm 48.16	2532.11 \pm 66.61

The *Murraya koenigii* aqueous extract (1mL) pretreatment for 7 days increased the mean cumulative concentration of buspirone from 55.87 \pm 8.81 to 1098.89 \pm 19.60 $\mu\text{g/mL}$ in duodenum (Fig. 1), 137.86 \pm 16.10 to 1165.54 \pm 83.99 $\mu\text{g/mL}$ in jejunum (Fig. 2), ileum in 165.23 \pm 7.87 to 712.01 \pm 43.13 $\mu\text{g/mL}$ (Fig. 3) and in colon the mean cumulative concentrations were increased from 176.70 \pm 13.76 to 1241.13 \pm 48.16 $\mu\text{g/mL}$ (Fig. 4) respectively.

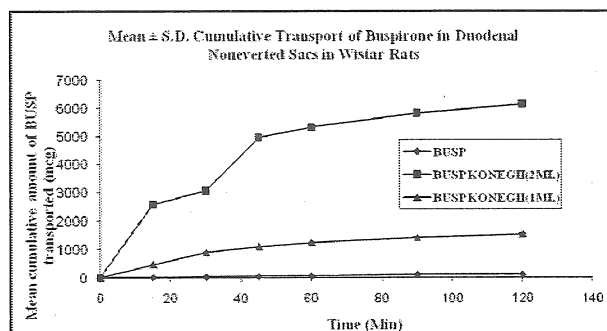


Figure 1. Effect of *Murraya koenigii* aqueous extract on transport of buspirone across duodenum

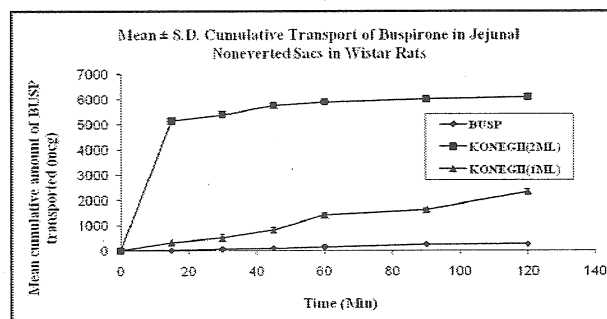


Figure 2. Effect of *Murraya koenigii* aqueous extract on transport of buspirone across jejunum

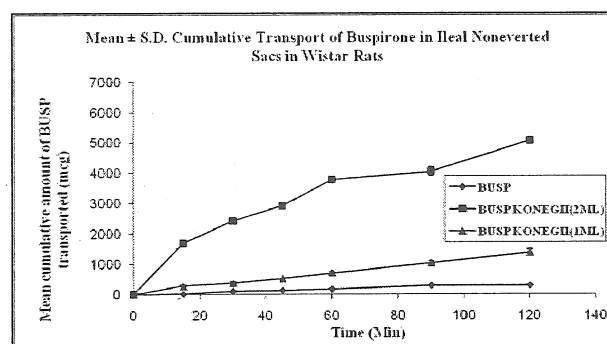


Figure 3. Effect of *Murraya koenigii* aqueous extract on transport of buspirone across ileum

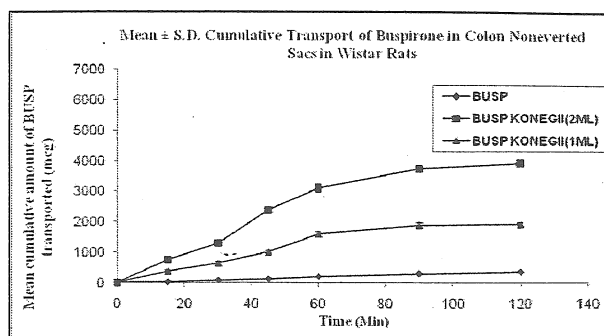


Figure 4. Effect of *Murraya koenigii* aqueous extract on transport of buspirone across colon

The *Murraya koenigii* aqueous extract (2mL) pretreatment for 7 days increased the mean cumulative concentration of buspirone from 55.87 ± 8.81 to 4682.67 ± 54.24 $\mu\text{g/mL}$ in duodenum (Fig. 1), 137.86 ± 16.10 to 5720.43 ± 60.23 $\mu\text{g/mL}$ in jejunum (Fig. 2), ileum in 165.23 ± 7.87 to 3319.33 ± 52.80 $\mu\text{g/mL}$ (Fig. 3) and in colon the mean cumulative concentrations were increased from 176.70 ± 13.76 to 2532.11 ± 66.61 $\mu\text{g/mL}$ (Fig. 4) respectively.

The transport of buspirone was increased 19.6, 8.4, 4.3 and 7.02 times after pretreatment with *Murraya koenigii* aqueous extract (1mL); 83.8, 41.49, 20.32 and 14.32 times after pretreatment with *Murraya koenigii* aqueous extract (2mL) compared to respective control, there was a statistically significant ($P < 0.005$) difference was observed.

The non-everted sac model was originally used to evaluate drug transport mechanisms (Kaul, and Ritschel 1981). Genty et al., (2001) compared the permeability values of some actively transported molecules and passively absorbed compounds through everted and non-everted sacs and found that the permeability was higher for actively transported molecules when the sacs were everted. The permeability of passive absorption drug diazepam remained the same whether the sacs were everted or not. These results suggested that the passive permeability of actively transported molecules can be determined through non-everted rat gut sacs (Kivisto et al., 1997).

However, Caco-2 would clearly overestimate efflux in both rat and human colon by 5- to 6-fold. This is interesting given that these cells are derived from colonic epithelium. One explanation of this is that in common with many tumors derived cell lines, Caco-2 may over express P-gp in relation to its parent tissue (Van Hille et al., 1996). The results from previous studies (Lamberg et al. 1998a and 1998b, Lilja et al., 1998) demonstrate that CYP3A inhibitors, verapamil, diltiazem, erythromycin, itraconazole and grape fruit juice, substantially increase the area under the curve (AUC) and the maximum concentration (C_{Max}) of buspirone in human plasma, presumably by inhibiting CYP3A mediated metabolic clearance. In addition a CYP3A inducer, rifampicin decreases the AUC and C_{Max} of buspirone in human plasma by 90 and 84 %, respectively (Kivisto et al., 1999). These observations strongly suggest that CYP3A isoforms play an important role in the metabolism of buspirone in humans. From the present study, it appears that pretreatment with *Murraya koenigii* aqueous extract 10 mL Kg^{-1} /po daily had strong effect on the intestinal transport of buspirone compared to *Murraya koenigii* aqueous extract was administered at a dose of 5 mL Kg^{-1} /po daily.

Buspirone is an azapirone anxiolytic agent that produces less sedation and impairment of psychomotor performance than do benzodiazepines. It has poor bioavailability due to extensive first-pass metabolism. The main constituents of the fruit oil were alpha-pinene (48.1%), beta-pinene (7.1%), myrcene (3.1%), beta-phellandrene (26.0%), gamma-terpinene (3.0%) and beta-caryophyllene (3.0%) (Mallavarapu et al. 2000). Tannins and terpenes present in the *M. koenigii* fruit may be acting as a CYP3A inhibitors (Yoshida et al., 2006).

Conclusion

Murraya koenigii aqueous extract pretreatment appear to have a significant influence on CYP3A4 mediated intestinal metabolism of buspirone. However, it is difficult to extrapolate our results, which were obtained in rats to humans.

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