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Research Article

A comparative study of release profiles of *Coccinia cordifolia* and *Catharanthus roseus* with standard antidiabetic agent using rat intestine

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Abstract

Matured leaves of *Coccinia cordifolia* and *Catharanthus roseus* were collected, dried and extracted with 95% ethanol. Solvents were evaporated and suspensions at a concentration of 40 mg/ml were prepared from the residues using phosphate buffer. The aim of the study is to elucidate the release pattern of these extracts in acidic and basic environment. 1 ml suspension of each plant was poured into the intestine fragment prepared from sacrificed rats. The filled pieces of intestine were bound vertically to the paddle of modified dissolution apparatus and rotated at 50 rpm in phosphate buffer incubation medium at pH 3 and pH 7.4. Metformin hydrochloride was used as the standard. Percent releases of *Coccinia cordifolia*, *Catharanthus roseus* and metformin hydrochloride were determined by analyzing the UV absorbance data at different time interval and the result obtained at pH 3 was compared with that of pH 7.4. Experimental result with these extracts and drug showed burst release initially followed by gradual release at pH 3.0 and the value of R^2 (correlation coefficient of percent release versus time) indicates that the release pattern was better maintained at pH 3.0 for coccinia and catharanths while that was better maintained at pH 7.4 for metformin hydrochloride.

Keywords: *Coccinia cordifolia*, *Catharanthus roseus*, metformin hydrochloride, release pattern, antidiabetic

1. Introduction

Coccinia cordifolia and *Catharanthus roseus* are two common herbs grow abundantly throughout the year all over Bangladesh. These plants are used for long time by human beings as antidiabetic agents without producing undesirable effects.
Ivy gourd or *Coccinia cordifolia* Linn. (Locally known as Telakucha) is cultivated in Southeast Asia for its edible young shoots and edible fruits[1]. The juice of the roots and leaves is considered to be a useful treatment of diabetes[2,3,4,5,6,7]. Researchers have also shown improvement in glucose tolerance of coccinia in patients with maturity onset diabetes[3,8]. The plant is also useful in reducing the serum total cholesterol and triglyceride levels[2,3,9].

The leaves of *Catharanthus roseus* (locally known as Nayantara) are used traditionally in various regions of the world including India, West Indies as well as Nigeria to control diabetes. The leaves of catharanthus has significant blood glucose lowering activity[2,10,11,12]. Several studies have demonstrated significant reduction in the levels of total cholesterol, triglycerides, LDL (low-density lipoprotein) and VLDL (very low-density lipoprotein) cholesterol using the leaves of catharanthus[2,10,12,13,14,15,16,17].

Although the use of herbal remedies for the treatment of diabetes mellitus has greatly declined in Europe and other Western nations since the introduction of insulin and oral hypoglycemic agents, rural communities of many developing countries still rely on the use of plant remedies to treat and/or manage diabetes mellitus[18]. Furthermore increasing reliance on the use of medicinal plants in industrial societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies.

Drug release is the foremost important factor to maintain the effective plasma concentration for the therapeutic point of view. It is obligatory for any drug delivery system to provide effective therapeutic concentration of the drug to the site of action and then maintain it all through.

Bioavailability represents the rate and extent of oral dose reaching the blood circulation which is controlled by solubility and dissolution rate of a drug in the intestinal fluid and permeability across the intestinal membrane, pre-systemic metabolism and efficiency of drug transporting system. It is very difficult to measure the accurate intestinal permeability for drugs and nutrients directly in human study and also unrealistic to predict the ability of a drug molecule to cross the intestinal barrier from simple physicochemical measurements such as pKa, molecular size and partition coefficient. Therefore, a number of *in vitro* and *in vivo* experimental models have been developed to determine the intestinal absorptive potential of a drug and the mechanism of absorption[19].

The absorption of drugs administering orally is a subject of intense and continuous investigation in the pharmaceutical industry since good bioavailability implies that the drug is able to reach the systemic circulation by mouth [20]. The intestine, in addition to the liver, is an important tissue that regulates the extent of absorption of orally administered drugs, since the intestine and liver are involved in first-pass removal[21,22]. The majority of drug absorption occurs at the small intestine because of the large surface area and also the presence of villi and

microvilli increases the absorptive area manifold[23].

To analyze the exact effects of a drug in a whole animal are complicated due to the possibility of observed consequence by acting at many targets at a time. The experiments are also complicated because the effect produced will depend partly on how the drug is administered, whether it reaches its sites of action immediately or only slowly over a long period. The action of the body on the drug is also important. The effects observed will depend upon the rate of excretion and metabolism of the drug and may be quite different from those observed with isolated preparations, where the drug is added in a known concentration at a known time to a small piece of tissue and washed out at a known time[24].

Pharmacological experiments designed to assay activity (i.e., the quantitative experiments) became very important in the 1920, because of the need to assay extracts of useful drugs, which could not be measured by any physical or chemical method. The estimation of the amounts of active drug constituents in plant or tissue extracts is still an important part of pharmacology in addition to compare the activities with known standards [25].

In our previous study we have compared the antihyperglycemic and hypolipidemic activity of the ethanolic extracts of *C. coccifolia* and *C. roseus*[2] and in the present investigation we have studied their release pattern in phosphate buffer incubation medium at two different pH.

2. Materials and Methods

2.1 Plant materials

Fresh leaves of *C. cordifolia* and *C. roseus* were collected from the Rajshahi University campus and were dried under shadow for several days. The dried leaves were grinded to a coarse powder. The authenticity of the *C. cordifolia* and *C. roseus* were identified by Mr. A.H.M. Mahbubur Rahman, Department of Botany, University of Rajshahi. Voucher specimens, collection # 33, dated 4/25/2002 for *C. cordifolia* and collection # 37, dated 10/30/2002 for *C. roseus*, were kept in the Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh.

2.2 Reagents

Metformin HCl was the generous gift sample from Square Pharmaceuticals Ltd. Pabna, Bangladesh.

2.3 Preparation of ethanol-extract

Dried leaves of *C. cordifolia* and *C. roseus* were soaked for 5-7 days in 95% ethanol with occasional shaking and stirring. Then they were passed through cotton filter. The collected filtrates were concentrated under atmospheric pressure below 45˚C to yield an ethanol-extract (yield 50.0 g; 5.0%).

2.4 Animals

Male albino rats weighing 200 ± 20g were collected from ICDDR,B, Dhaka, Bangladesh. They were kept in cases and maintained in well-ventilated room under conditions of natural light and dark cycle. They were fed with standard diet and water *ad libitum*. The animals were fasted 24 hours allowing access only to water and were deprived of food and then sacrificed to collect the intestine.

2.5 Preparation of phosphate buffer

Phosphate buffer was prepared using 2.38 gm of disodium hydrogen orthophosphate, 0.19 gm of potassium dihydrogen orthophosphate and 8.0 gm of sodium chloride in sufficient water to produce 1000 ml phosphate buffer (BP 1988). pH of the buffer was adjusted to 7.4 and 3.0, respectively just before use.

2.6 Determination of λ max

λ max of metformin hydrochloride, and ethanolic extracts of *catharathus roseus* and *Coccinia cordifolia* were determined against buffer 7.4 and 3.0 pH. The λmax of metformin hydrochloride, and ethanolic extracts of *catharathus roseus* and *Coccinia cordifolia* were 233, 205 and 207, respectively at 7.4 pH buffer solution, while that of 233, 218 and 216 for metformin hydrochloride, and ethanolic extracts of *catharathus roseus* and *Coccinia cordifolia*, respectively at pH 3.0.

2.7 Experimental solutions

Metformin hydrochloride was taken as the standard for this experiment. Two solutions were prepared as phosphate buffer of pH 7.4 and 3.0. Sample solutions were also prepared with ethanolic extract of *Cathararthus roseus* and *Coccinia cordifolia* in phosphate buffer at pH 7.4 and 3.0. The concentration of all the standard and sample solutions was 40 mg/ml. The factors that were considered while preparing the dose include that the extracts were in crude form, the average weight of our rats were 200 gm and the dose of metformin hydrochloride is 2000 mg maximum per day for adult.

2.8 Experiment design

A rat was sacrificed anesthetizing with flurothane. The abdomen was opened and a length of ileum was removed and placed in a petridish containing phosphate buffer at 4˚C to keep the intestine fresh. The rat was kept fasted for about 24 hours so the intestine was found clear enough to use directly. Three fragments were then cut from the intestine each about 6 cm long. One end of the fragment was tied. 1 ml (i. e. 40 mg) of each sample and standard solutions were poured into the intestine through the other end and then tied. After pouring the solutions the fragments were tied vertically to three paddles of the modified dissolution apparatus using thread. The fragments of intestine were handled with great care using finger rather than gripping with forceps to avoid damaging of the gut muscle. The paddles were then immersed into the basket of the modified dissolution apparatus containing phosphate buffer (pH-7.4) 900 ml each. The paddles were allowed to rotate at 50 rpm and the temperature of the dissolution tester was kept 36±1°C. Samples were collected from each basket after 10, 20, 30, 60, 90, 120 and 180 minutes from the starting. Samples were collected in a volume of 5 ml and compensated with fresh medium kept at 37°C immediately each time.

Absorbances of samples were taken at respective λmax of Metformin hydrochloride, *Coccinia cordifolia* and *Catharanthus roseus* (233, 207 and 205, respectively). This experiment was done for 6 times repeatedly, and another 6 times using phosphate buffer of pH 3.0. In later 6 experiments absorbance was taken at 233, 216 and 218 for Metformin hydrochloride, *Coccinia cordifolia* and *Catharanthus roseus*, respectively that are at their respective λmax.

2.9 Statistical analysis

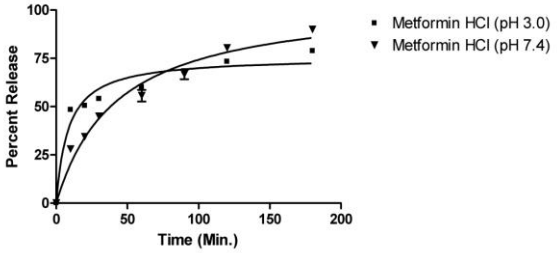
Percent release of the drugs and extracts were calculated from their UV absorbance data using reference standard. Correlation coefficient (R^2) of % release versus time was determined. Statistical calculations and the graphs are prepared by Graph Pad Prism (version 3) computer program (GraphPad Software SanDiego,CA, USA).

3. Results

Percent release of metformin hydrochloride and ethanolic extracts of *Coccinia cordifolia* and *Catharanthus roseus* were determined by analysing their UV absorbance data. The experiment was performed at two different pH (3.0 and 7.4) to compare the release patterns of the plant extracts and the standard.

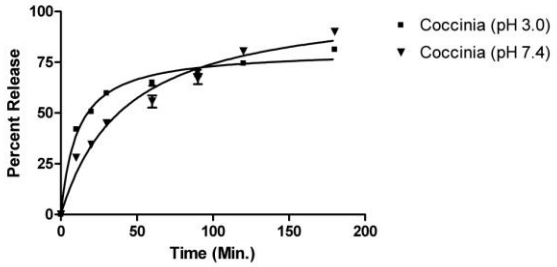
In figure 1 the percent release of Metformin hydrochloride is plotted against time at two different pH (3.0 and 7.4) which are 78.9 ± 0.9 % at pH 3.0 and 84.9 ± 1.2 % at pH 7.4 after 180 minutes.

Figure 1: The percent of release of metformin hydrochloride at pH 3.0 (■) and 7.4 (▼). The values are presented as mean ± SEM of 6 experiments.



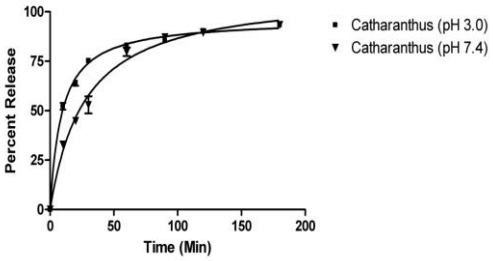
In Figure 2 the percent release of ethanolic extract of *Coccinia cordifolia* is plotted against time at pH 3.0 and 7.4. The release of the extract is 81.2 ± 0.9% at pH 3.0 and 89.9 ± 1.4% at pH 7.4 on 180 minutes treatment.

Figure 2: The percent of release of ethanolic extract of *Coccinia cordifolia* at pH 3.0 (■) and 7.4 (▼). The values are the mean ± SEM of 6 different experiments.



Similarly figure 3 shows the releases of the ethanolic extracts of *Catharanthus roseus* at different pH which are 93.3 ± 0.4 and 93.3 ± 1.2% at pH 3.0 and 7.4 respectively after treating for 180 minutes.

Figure 3: The percent of release of ethanolic extract of *Catharanthus roseus* at pH 3.0 (■) and 7.4 (▼). The values are mean ± SEM of 6 different experiments.



4. Discussion

We have demonstrated the release pattern of metformin hydrochloride at pH 3.0 and 7.4 in the phosphate buffer incubation medium at different time intervals. Percent release of metformin hydrochloride after 180 minute was 78.8 and 84.9% at pH 3.0 and pH 7.4 respectively. Correlation coefficient (R^2) of percent release versus time was 0.9522 and 0.9564 at pH 3.0 and pH 7.4 respectively. The value of R^2 at both experimental pH is close to unity, which implies that the release was maintained at monophasic exponential, but the release rate was higher at intestinal pH, though this drug showed initial burst release followed by gradual release at pH 3.0 (Fig-1).

The release study of *Coccinia cordifalia* extract was performed in phosphate buffer incubation medium at pH 3.0 and 7.4. The percent of release of the extract was measured at different time interval and that was 81.18 and 89.9% at pH 3.0 and 7.4 respectively after 180 minutes treatment. Correlation coefficient (R^2) of percent release versus time was 0.9790 and 0.9532 at pH 3.0 and 7.4, respectively. However, the R^2 value at pH 3.0 (0.9790) is nearer to unity which implies that release is better maintained at pH 3.0 with initial burst release followed by gradual release (Fig-2).

Similarly the release of *Catharanthus roseus* extract was performed in phosphate buffer incubation medium at both experimental pH and the percent of release was measured at different time interval. After 180 minute the percent of release was 93.281 and 93.342% at pH 3.0 and 7.4 respectively. However, the correlation coefficient (R^2) of percent of release versus time was 0.9915 and 0.9731 at pH 3.0 and 7.4, respectively. The R^2 value, 0.9915 is nearer to unity which implies that release is better maintained at pH 3.0 with initial burst release followed by gradual release (Fig. 3).

Coccinia cordifolia and *Catharanthus roseus* were selected in our study for their use in diabetes. Because of the significance of the intestine as an important first-pass organ, high-throughput *in vitro* systems have been developed to assess the importance of intestinal absorption. The present study with the extracts of C. cordifolia and C. roseus provides the evidence that the release pattern was better maintained at pH 3.0 for coccinia and catharanths while that for metformin hydrochloride release pattern was better maintained at pH 7.4. Thus, this newly established method would be a powerful tool for the analysis of drug release pattern as well as for drug development in the native tissue environment.

The research outcome in this study based on the absorbance of the extracts, which mainly originated from the absorbance of the compound, exists as highest concentration. Detail study is necessary whether the compound is responsible for lowering blood glucose and lipid.

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