INTRODUCTION

For years, methods to improve the efficacy of medications have been widely studied. It is known that the efficacy of a drug depends on its dosage form and route of administration [1]. Effervescent tablets are one of these forms that have drawn the attention of many for several reasons. Vitamin C tablets are probably the most well-known effervescents. However, several other medications are also made in this form nowadays [2]. These include Aspirin, antacids, iron, calcium and glucosamine supplements. These are all used in treating a wide range of conditions, from arthritis, pain and inflammation, to stomach and bowel problems, wounds, allergies and osteoporosis [3].

Acetaminophen (N-acetyl para aminophenol, 4-hydroxyacetanilide) is absorbed quickly if administered orally [4]. However, the first pass effect reduces its access to proper circulation. It is an over-the-counter, non-narcotic drug which is widely used to relieve pain and reduce mild to moderate fevers. Although its mechanism of action has not been precisely worked out, studies have shown that the inhibition of central prostaglandin synthase plays an important role in the process. Acetaminophen does not inhibit the production of prostaglandins in the gastric mucosa [5], however, and, consequently, it can be helpful for patients with gastrointestinal disorders. It is also used as a part of combination therapy with other analgesics and anti-inflammatory agents. Several methods have been employed to make the absorption of acetaminophen tablets faster [6,7]. These include increasing...
the disintegration of the tablet, increasing the solubility of the drug using amino acid or alkali metal salts, and even adding sorbitol or antacids to acetaminophen tablets. The pharmacokinetics of various dosage forms of acetaminophen have been also broadly studied [8]; however, few studies have provided information on the amount of acetaminophen absorption in capsule formulation or the comparison of this amount between different formulations. Capsule and effervescent formulations are prepared of replace with can change acetaminophen to a more hydrophilic and more soluble powder [9]. The aim of this study was to assess the main pharmacokinetic properties of 3 formulations of acetaminophen (capsules, effervescents and tablets) based on the previously described acetaminophen powder and also to compare these features with all three accessible brand formulations of acetaminophen (2 conventional forms of solid tablets, effervescents and capsules).

MATERIALS AND METHODS

This study has been approved by the ethics committee of Babol University of Medical Sciences (Babol, Iran) and recorded in the data bank with a registration number. All subjects signed a written informed consent.

Materials

HPLC grade acetonitrile and water were purchased from Daejung (Daejung Chemicals Ltd., Korea). 4’-Methylacetophenone and Acetaminophen (>99.99% purity) were purchased from Merck KGaA, Darmstadt, Germany). Purified de-ionized water was prepared using the Milli Q50 water purification system (Millipore, USA).

High-performance liquid chromatographic conditions

The HPLC system was equipped with UV/VIS variable wavelength detector (KANUER, Germany), degasser article Nr. A5328 (KANUER Corporation, Germany). Acetophenone was used as internal standards (IS). Acetaminophen and (IS) were extracted using a mobile phase of acetonitrile and 0.1% acetic acid in water (20/80), via C18 column (4.6 mm x 25 mm) and UV detector (wavelength = 240 nm). Chromatographic resolution of Acetaminophen in serum was achieved on a (4.6 mm x 25 mm) C18 column. Samples were then injected into HPLC with a syringe loading injector fitted with a 20 µl loop.

Preparation of Standard solutions

An exact quantity of acetaminophen (5 mg) was transferred into a 5 ml volumetric flask. Approximately 2.5 ml of acetonitrile with 0.1% acetic acid (mobile phase) was then added and dissolved. To obtain a final concentration of 1.0 mg/ml, the solution was brought to volume by the mobile phase solvent and fully mixed. The prepared stock solution was kept at 4°C in a falcon vial. Standard solutions were directly injected to HPLC (Figure 2).

Linearity

The calibration curves were produced with four concentrations ranging from 5 to 40 µg/ml of acetaminophen. Each concentration level was prepared and analyzed three times. Thereafter, calibration curves were produced by plotting peak area response versus the concentration of compounds. The least square regression method was used for evaluation of linearity. The coefficient variation (CV) for this analysis was 3.8%.

Figure 1. Least square regression method used for evaluating linearity

The study procedure

We enrolled 30 healthy volunteers in the study replace with into our study: 15 men and 15 women, with a mean (±SD) age of 21 (±2). Participants were then placed within 3 groups, each containing 10 subjects, all of whom being prohibited to use acetaminophen or other analgesics one day before the experiment.

Preparing samples

For each participant, a sample of blood (5 ml) was collected using an angiocatheter. The sample was obtained once before taking the drug by the subjects, and at 0.5, 1, 2, 4, 8 h after taking it, respectively. Blood samples were then collected in labeled, heparinized glass tubes and put into a 37°C water bath in order to separate serum contents. Thereafter, the samples were centrifuged in a refrigerated centrifuge (4°C) for 10 min at 3000 rpm. They were then immediately transferred to a freezer and kept under -20°C for later assays. To extract acetaminophen from the serum, 100 µl of the serum sample was transferred to a 2 ml vial containing 20 µl 4-methylacetophenone as internal standard. A volume of 500 µl of methanol was added to the sample afterwards. The vial was then vortexed for 1 min and the solution that remained on top was separated using a sampler. Subsequently, 100 µl of solvent was stored with an open cap in a 4°C fridge. A volume of 20 µl of final extracted solution was directly injected to HPLC (Figure 2).

The pharmacokinetic (PK) parameters such as absorption rate constant (Ka), clearance, half-life and volume of distribution of acetaminophen in three groups were calculated based on one-compartment kinetics using P-Pharm software (10,11). According to data distribution (Table 1), the results were analyzed using analysis of variance. The difference of the PK parameters between three groups was considered statistically significant at p < 0.05.

Statistical analysis

According to the results, On-way ANOVA followed by tuky post-hoc test was used. The differences of the PK parameters of three groups were analyzed and considered statistically significant at P<0.05.
Table 1. Comparison of the PK parameters of three groups receiving Acetaminophen

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Cl (L/min)</th>
<th>V/F (L)</th>
<th>Ka (1/min)</th>
<th>t1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eff</td>
<td>1.38</td>
<td>2.29</td>
<td>1.20</td>
<td>1.11</td>
</tr>
<tr>
<td>Cap</td>
<td>0.11</td>
<td>0.11</td>
<td>0.13</td>
<td>24.64</td>
</tr>
<tr>
<td>Tab</td>
<td>0.16</td>
<td>0.25</td>
<td>0.16</td>
<td>27.47</td>
</tr>
</tbody>
</table>

Rtmax: maximum time to maximum concentration, Cmax: maximum concentration, MIN: minimum concentration, MAX: maximum concentration, F: folding of minimum concentration or MAX divided by MIN, Eff: effervescent, Cap: capsule, Tab: tablet

RESULTS

Acetaminophen concentrations

Comparison of acetaminophen concentrations shows a statistical difference between the three groups (p < 0.05); Table 2 and Figure 3 show serum concentrations of effervescents in comparison with the other two groups. In the effervescent group, the highest concentration was observed at 60 min after dosing. The concentration then increased rapidly to the maximum level after 60 min, decreased gradually and reached its lowest point at 480 min. The maximum and minimum concentrations of acetaminophen were 15.24 and 1.49 µg/ml for the effervescent, 11.29 and 2.08 µg/ml for the capsule and 8.73 and 2.64 µg/ml for tablet forms, respectively (Table 2).

Table 2. Comparison of the Area under the curve of three groups receiving Acetaminophen

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Observed plasma concentration (µg/ml)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Eff</td>
<td>6.61±2.41</td>
<td>11.29±3.94</td>
</tr>
<tr>
<td>Cap</td>
<td>8.74±2.49</td>
<td>7.43±1.37</td>
</tr>
<tr>
<td>Tab</td>
<td>7.35±2.80</td>
<td>4.67±1.49</td>
</tr>
<tr>
<td>Eff</td>
<td>2.64±0.85</td>
<td>2.08±1.70</td>
</tr>
<tr>
<td>Cap</td>
<td>47.04</td>
<td>53.11</td>
</tr>
</tbody>
</table>

Standard curve

To determine the concentration of acetaminophen in serum, a standard curve was plotted with standard solution of the drug containing 100 µg/ml of acetaminophen prepared in methanol. Of note, 4'-methylacetophenone was added as internal standard for all concentrations (Figure 1). Standard peaks of acetaminophen of 5, 10 and 20 µg/ml and internal standard (10 µg/ml) were prepared. A standard curve was obtained using the peak area under the curve (Figure 2). Moreover, R2 index (linearity) was depicted in the chart.

Figure 2. The Sample peaks of acetaminophen, (3.4 min) (A) standard acetaminophen (10 µg/ml); (B) serum sample after 2h acetaminophen administration; (C) serum Sample after 1h acetaminophen administration

Data analysis with P-Pharm

We used P-Pharm software to model and analyze the obtained data on serum concentrations of the drug. According to the modeling of serum concentrations of acetaminophen versus time, the results of oral administration of three-compartment kinetics on the PK modeling are shown below:

A) Concentration versus time curve in tablet group: Figure 3A shows the correlation between concentrations versus time for any subject in the tablet group. This figure shows only two scattered levels at 120 min.

B) Concentration versus time curve in capsule group: Figure 3B shows a correlation replace with regression between concentrations versus time of the individuals in the capsule group. This figure demonstrates scattered concentrations at 60 and 240 min after drug administration. The maximum concentration of acetaminophen in serum samples was recorded 60 min after oral administration (Figure 3B).

C) Concentration versus time curve in effervescent group: Figure 3C indicates a correlation replace with regression between concentrations versus time of the subjects in the effervescent group. In this figure, scattered concentrations are visible at 60 min after drug consumption.
Comparison of oral bioavailability of acetaminophen tablets, capsules and effervescent dosage forms in healthy volunteers

**Figure 3.** The relationship between acetaminophen concentrations vs. time in each subject (individual fitting) in A Tablet, B Capsule and C Effervescent groups after PK modeling based on Three compartment Oral administration kinetics. A Krukenberg tumor is a metastatic adenocarcinoma of for proper treatment

**Figure 4.** Mean of plasma concentration - time profiles of acetaminophen in the three groups of the study after a single 500 mg oral dose of acetaminophen from different dosage forms

**DISCUSSION**

Salt formation has a key role to increase solubility and absorption of acetaminophen [10] without any changes in chemical structure and pharmacological properties. Among different formulations of acetaminophen, the effervescent dosage form has a quicker absorption (Greater $C_{max}$ and lower $T_{max}$) (Table 2). The area under the plasma concentration curve, however, shows that tablet and capsule forms were absorbed much more than the effervescent during the first hour of administration and the AUC of effervescent group is significantly higher than the two other formulations.

When the goal is to relieve pain in a faster and more efficient way, it is better to use soluble or quickly-absorbed formulations because delayed absorption or low concentrations of the drug may result in treatment failure. Another issue that has always been of great importance for pharmacists is to prevent drug alterations that occur in the GI system [11]. Such changes may occur due to the low pH of the stomach and food/drug interactions, causing the drug to be inactivated. With their buffering properties, effervescents increase gastric pH and therefore prevent active drug forms from dissociation and inactivation [12]. This buffering feature (carbonation) of effervescents helps the stomach to empty itself quickly. It usually takes 20 min for gastric components to reach the small intestine, therefore, the same amount of time is required for activated drugs to reach maximum absorption [13].

In all the 3 groups, we observed that the serum concentrations of the drug reached their maximum at the first hour after administration. Our study also found that capsules and effervescents equally reached the maximum concentration for acetaminophen, while it took 120 min for tablets to reach this concentration after a single dose of the drug. In addition, effervescents had the highest serum concentrations during the first hours of administration – more than 2.5 times greater than tablets.

Studies have shown that compared with tablets, effervescents better improve the absorption rate of several activated drugs (e.g caffeine and disulfiram), simply because the CO$_2$ resulting from acid-base reactions can increase their permeability [14]. Given that the drug component of effervescents enters the GI system as a recently produced solution [15],

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they are absorbed at a higher rate than other formulations. In addition, when effervescents dissolve in water, the pH of the final solution causes the drug to transit faster from stomach to small intestine. Effervescents also last longer than liquid drug forms such as syrups or suspensions [16].

CONCLUSIONS

The three reasons mentioned above indicate that effervescents have a quicker effect compared with tablets. We can claim that the absorption of the drug component of effervescents is faster than tablets and a faster absorption may suggest a faster effect.

ACKNOWLEDGMENTS

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

REFERENCES