Research Article

ORAL ADMINISTRATION OF A FLAVANOID QUERCETIN IN RAT PLASMA ON BIOAVAILABILITY STUDY ANALYSIS BY HPLC

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Abstract

The present study has been performed to assess the role of quercetin, a dietary flavonoid on bioavailability study in rat plasma. The bioavailability study was achieved by HPLC to predict the pharmacokinetic parameters of quercetin after oral administration at the dose of 50 mg/kg b.w to male Wistar rats, blood samples were collected and separated at different time intervals (0, 6, 12 and 24 hrs). Separation was achieved on Eclipse XDB-C18 column (200 × 4.6 mm) using methanol: acetonitrile (30: 70, v/v) as a mobile phase at a flow rate of 1.0 ml/min and data were analyzed using one-way analysis of variance (ANOVA). From the calibration curve, the amount of quercetin was calculated. In plasma sample, the retention time of quercetin was 2.34 and produced significantly different pharmacokinetic parameters in Tmax, Cmax, half life (t1/2) and area under curve (AUC). Our results demonstrate that the quercetin in the pharmacokinetic study could improve the ability to illustrate their bioavailability, understand the mechanism of drug action and help to promote the drug development phase.

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1. Introduction

Phytochemicals are produced in plants and it protects the plant against insect invasion, infection, ultraviolet light damage and diseases due to its natural biological action. Plants also derive characteristics of colour, flavour, and odour from phytochemicals. Flavonoids constitute a class of phytochemical are divided into different subclasses and the contributions of flavonoids to health are based on their molecular structure (Nijveldt et al., 2001). Flavonoids are non-energetic and are not considered as indispensable as are vitamins, yet the supply of some of them, or maybe of a complex mix, may have a positive effect on health. Two recent epidemiological studies support the view that flavones and flavonols protect against cardiovascular mortality (Hertog et al., 1995; Rimm et al., 1996). Also, they have been shown to inhibit the growth of various cancer cell lines in vitro, and to reduce tumor development in experimental animals (Manach et al., 1996).

Quercetin is a flavonol and is generally originate in several plant based foods such as apples, onions, berries, broccoli, red wine, red grapes, bark roots, citrus fruits, flowers, and tea (Nutrient et al., 2001). Quercetin supplements are marketed to the people as on other therapy for treating allergies, arthritis, gout, eye disorders, asthma, hypertension, bacterial infections, and neurodegenerative disorders, quercetin as an antihypertensive agent and also decrease blood...
pressure. Quercetin is a versatile molecule among numerous pharmacological properties as well as antioxidant, antimicrobial, anti-viral, anti-inflammatory, anticancer, antiobesity agent neurological, cardiovascular, hepatoprotective and protective of the reproductive system (Jan et al., 2010).

The quercetin bioavailability is dependent on the form of quercetin is resulting from the glycoside forms in better absorption than quercetin aglycone (Manach et al., 2005) Other factors like intestinal flora and dietary mechanism could affect the bioavailability of various isoforms of quercetin. Such as rutin and pectin supplementation results that increased quercetin level in mice plasma concentrations (Tamura et al., 2007). Same results were observed in rats fed a diet elevated in pectin that was orally administered a single (50 mg/kg) dose of quercetin (Nishijima et al., 2009). In pigs, the quercetin aglycone and quercetin- 3-O-glycoside both are enhance absorption in the dietary fat (Lesser et al., 2004; Lesser et al., 2006). Some studies investigated the ability of previous polyphenolic compounds to influence quercetin absorption while, the co-supplementation of epigallocatechin gallate and quercetin could raise epigallocatechin gallate absorption in rats (Kale et al., 2010). The aim of the current study examined to analyse the bioavailability study of quercetin in plasma by HPLC.

2. Materials and Methods

Chemicals

All the chemicals used in the experiments were of HPLC grade. Quercetin was procured from Sigma Aldrich, (St. Louis, MO, USA) and the solvents were procured from Hi-media Laboratories Pvt, Ltd., Mumbai, India.

Experimental animal

Male Wistar rats (180 – 200 g) from Central Animal House, Annamalai University, Tamil Nadu and India were maintained at 24 ± 2 °C on 12 hrs light/dark cycles. The rats were nourished with standard diet pellets (Hindustan Lever Ltd., Bangalore, India) and water ad libitum. Investigations were performed conferring to the ethical norms of the Institutional Animal Ethics Committee of Annamalai University (Approval No. 994, dated 02.05.2013). The rats were acclimatized for a week before starting the experiments.

Experimental Study

The developed HPLC method was used in a pharmacokinetic disposition study after oral administration of quercetin (50 mg/kg body weight) to male Wistar rats (120 – 180 g). Venous blood samples were collected at 0, 6, 12 and 24 hrs in heparinised tubes. Blood samples were immediately centrifuged at 3000 rpm for 5 min and harvested plasma samples stored at -20 °C until analysis. The standard solution was prepared by stock solution of quercetin (1 mg/ml) in methanol. These solutions were spiked in to drug free rat plasma sample to determine the recover, precision, accuracy and detection limit of the HPLC method.

Sampling procedure

Plasma samples (0.1 ml) were shaken with 1.0 ml methanol for 2 min and centrifuged at 3000 rpm for 10 min. The methanol extract was transferred to dry tube. The procedure was repeated twice and the methanol extracts collected were dried at 40 °C under a nitrogen stream. The residue was dissolved in 100 μl methanol and then injected into the chromatographic system. Quercetin content was analysed by reverse phase - HPLC with a UV detector and a vacuum degasser run by Auto chro - 3000 software (Acme 9000 series; Young Lin,USA). Chromatographic separation was achieved by using a reverse – phase C18 column (150 mm and 4.6 mm, pore size 5 μm). The test was carried out by injecting 25 μl of mixture of standard solution at assay concentration of quercetin. The mobile phase consisted of 73 % methonal and water (99.5:0.5) (solvent A) and 27 % acetonitrile (solvent B), pH adjusted to 3.64 with glacial acetic acid. The separation was performed under an isocratic condition with a constant flow rate 1.5 ml/min, column temperature 25 °C and the detector wavelength 347 nm. The experiment was performed three times and the mean was used for
the calculations. The data was analyzed by linear regression.

**Statistical Analysis**

The results were presented as mean ± SD. One-way analysis of variance (ANOVA), followed by Duncan’s multiple range test (DMRT) was done (SPSS software version 13.0) and a p-value of <05 was considered significant.

3. Results

**Bioavailability of quercetin by HPLC**

HPLC chromatogram of quercetin standard and plasma from rats at different time intervals (0, 6, 12 and 24 hrs), after oral administration of quercetin at dose of 50 mg/kg body weight (Figure 1 and 2) The oral administration of free quercetin in rats, The retention time of quercetin was detected at 2.34 and the maximum plasma concentration (Cmax) achieved was 10.26 ± 0.08 µg/mL in 6 hours, Tmax was achieved at 6, t1/2 was 6.02 ± 0.36 and AUC was calculated at 18 ± 0.84 (Table -1).

**Discussion**

The present study demonstrates noticeable differences in the rate of absorption and of appearance of quercetin in blood plasma, when provided as quercetin. When rats were fed semi-purified diets containing either forms of this flavonol, quercetin was more rapidly absorbed than the aglycone moiety of rutin. Furthermore, quercetin appeared in detectable amounts in the blood circulation, long before the bolus had reached the cecum. By contrast, in rats fed diet, quercetin metabolites could be found in blood plasma. Thus, with a quercetin diet, the possibilities of absorption of this flavonol appear greater, since quercetin is available for digestive absorption both in the small intestine and in the large bowel. During the post absorptive period, whilst absorption in the small intestine was declining (due to transit of digesta and dilution of remaining digesta by endogenous materials), flavonol absorption took place in the large intestine.
50 mg/kg of quercetin in different formulations. The peak area of quercetin was measured when sample working solution was injected. Plasma concentration declined rapidly after 6 hours in rats, which indicating that the drug was distributed and metabolized rapidly. Manach et al., (1999) reported that quercetin is quickly metabolized by the intestinal enzymes and strongly bound to albumin, which might delay their emigration and so as to preferentially excrete in bile (Jain et al., 2013). The various concentration of

Table -1: Pharmacokinetic parameters of quercetin (50 mg / kg bw) in male Wistar rats after oral administration

<table>
<thead>
<tr>
<th>PK Parameters</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Cmax</td>
<td>10.26 ± 0.08</td>
</tr>
<tr>
<td>Tmax</td>
<td>6</td>
</tr>
<tr>
<td>t1/2</td>
<td>6.02 ±0.36</td>
</tr>
<tr>
<td>AUC</td>
<td>18 ±0.84</td>
</tr>
</tbody>
</table>

Cmax = Maximum plasma concentration, Tmax = Time for maximum plasma concentration, AUC= Area under plasma concentration time curve, t1/2 = Elimination half life. Values that are not sharing common superscript in the same column differ significantly at P < 0.05 was performed by one-way analysis of variance.

Figure -2: Plasma concentration-time profiles of Quercetin after oral administration in rats at dose of 50 mg / kg bw

Our present study showed that HPLC chromatogram of quercetin standard and plasma from rats at different time intervals (0, 6, 12 and 24), after oral administration of quercetin at dose of 50 mg/kg body weight. The oral administration
of free quercetin in rats, The retention time of quercetin was detected at 2.34 and the maximum plasma concentration (Cmax) achieved was 10.26 ± 0.08 µg/mL in 6 hours, Tmax was achieved at 6, t1/2 was 6.02 ± 0.36 and AUC was calculated at 18 ± 0.84 this is indicating that the quercetin was distributed and metabolized rapidly. Elsewhere, it is reported that quercetin is quickly metabolized by the intestinal enzymes (Manach et al., 1999).

4. Conclusion

The bioavailability study of quercetin (50 mg/kg b.w) produced extensively efficient pharmacokinetic parameters in Tmax, Cmax, half life and AUC. This kind of pharmacokinetic studies of active compounds could improve the ability to illustrate their mechanisms of action and help to promote the drug development phase in to lead development.

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5. References


