Bioavailability of Ellagic Acid in Plasma of 20 Healthy Volunteers Using HPLC Technique.

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Abstract

Ellagic acid (EA) and hydrolysable ellagitannins (ETs) were extracted from white flesh pomegranate; the ellagic acid was implicated with potent antioxidant, anticancer and antiatherosclerotic biological properties. ellagic acid act as excellent scavenger for chemical causing cancer , It form a layer on DNA to prevent free radicals damaging

The concentration –time curve was constructed after administration of 80 mg ellagic acid to 20 healthy volunteers (14 male and 6 females) . The calibration curve for quantification of EA was linear (r2 = 0.9998) over concentration range from 400 to 6.25 ng/ml. the maximum concentration of ellagic acid (C max) was achieved in plasma of volunteers after 1 h (T max). the mean serum elimination half life was about 6.21 ± 1.35 h . The recovery of ellagic acid in plasma at different concentration from 50-400 ng/ml were between 101-117 % .

Keywords: Bioavailability ، Ellagic Acid and Plasma.

قياس التوافر الحيوي لحامض اللاجيك في مصل الدم ل 20 من المتطوعين بتقنية السائل الكروموتوغرافي عالي الاداء

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الخلاصة

حامض اللاجيك ومادة الاجنين المتحلل من مستخلص الشحم الابيض للرمان له فعالية واسعة كمادة مضادة للاكسدة- مضادة للسرطان، مضادة لتصلب الشرايين، اضافة الى ان حامض اللاجيك يعمل على قنص المواد الكيمياوية المسببة للسرطان ويعمل طبقة رقيقة على الحامض الرايبوي لمنع تأثير الجذور الحرة.

قيست علاقة التركيز بالزمن بعد اعطاء 80mg من حامض اللاجيك الى 20 متطوعين اصحاء (14 ذكر و8 اناث). كما اشارت النتائج الى ان قياس حامض اللاجيك اعطى دالة خطية للتراكيز المحصورة بين (400-6.2 ng/ml) و اعلى تركيز لحامض اللاجيك بلازما الدم للمتطوعين وصلت خلال ساعة واحدة و زمن النصف لطرح حامض اللاجيك تم الوصول اليه خلال 6.21±1.35h ساعة ، النسبة المئوية لاستخلاص حامض اللاجيك المسترجع من بلازما الدم وكانت بين 101-117%.

الكلمات المفتاحية: التوافر الحيوي ، حامض اللاجيك و مصل الدم.

Introduction

The extracted polyphenols from various plants play an important role in human nutrition and are implicated with numerous biological properties including, anticancer, ontioxidant, anti-inflammatory, and anti-atherosclerotic activities. (Cerda, *et.al.*, 2003) Among these phytochemicals, ellagic acid (EA), which was highly available extract in white flesh pomegranate, either free ellagic acid, as EA or bound as ellagitannins (ETs) (Amakura, 2000).

The absorption, bioavailability and pharmacokinetics of EA administered orally have not been adequately investigated. (Aviram, *et al*., 1986).

Ellagic acid has been reported to have antiviral activity and provide high protective against cancers of colon, lung, esophagus and cervical cancer. (Boukharta, *et al*., 1992). The bioavailability and pharmacokinetic studies in human are necessary to determine the effect of these bioactive dietary polyphenols, apart from being prevalent in foods, are also commonly used as botanical ingredients in dietary and herbal supplements. (Navindra, *el al.*, 2004).

The previous knowledge on the bioavailability of EA and ETs is confined to animal studies with rats and mice (Smart, *el al*., 2000). When mice were given ETs (from raspberries or pomegranates at 600 mg/kg body weight,), EA was detected in the urine (0.05% of dose) as a result of absorption and metabolism of ETs (Belal, *et al*., 2009.

However, virtually no EA was recovered from the blood or tissues of mice fed for 1 week on a diet containing 1% EA (Cerda, *et al*., 2003 and Teel, *et al.*, 1988).

Following oral administration of EA to rat, 10% of the dose was excreted and detected as EA metabolites in urine and faeces (Smart, et al.,2000), (Borges *et al*., 2010).

The low levels of free EA in plasma have been attributed to its low solubility in water (Lei *et al*., 2003)..

Furtheremore may also be due to its extensive metabolic transformation and degradation prior to absorption. In addition, EA has been reported to bind irreversibly to cellular DNA and proteins which may also account for its limited transcellular absorption (Gil, *et al*., 2001).

The poor absorption of EA has been reported to impact it's in vivo anti-tumorigenic activity since it is possible that sufficient levels are not present in plasma or target cells after oral administration. (Ayrton *et al*., 1992 and Whitley, *et al*., 2003).

In this study we investigated the bioavailability and pharmacokinetics of EA in plasma of 20 healthy volunteers after oral single dose administration of 80 mg capsules to determine the concentration of EA in ther body.

Materials and Methods

Twenty, nonsmoking, healthy volunteers (14 males, mean age ± SD, 32.5 ±4.5 years; weight, 65.5 ± 6.5 kg; height, 168.5 ±4.0 cm) and (6 females, mean age ± SD, 28 ± 4.5 years; weight, 54.5 ± 4.5 kg; height, 162.5 ±5.5 cm) in two groups took part in this study. All volunteers gave written informed consent after they had received detailed instructions about the study performance of ellagic acid. All volunteers gave written informed consent after they had received detailed instructions about the study performance of ellagic acid. All volunteers were in good physical health according to physical examination, hematological and urinary laboratory tests. These valunteers did not take any other medications for at least 1 week prior to and throughout the study. Each valunteers fasted overnight before the experiment. Ellagic 80 mg

capsules were swallowed with 150 ml water.
A light, normal lunch consisting of cheese, bread, tea and water was given to all valunteers 4 hours after dosing.

Blood Sampling

The blood samples had been drawn from each volunteer after oral dose administration of 80 mg ellagic acid capsules, according to the randomization schedule, with 150 ml drinking water.
Blood samples (3 mL) were taken via an indwelling venous cannula at the following times: before drug administration (0 hr), and at 0.3, 1, 2, 3, 4, 6, 8, 10 and 12 hr after administration, the collected plasma transferred to labeled tubes. The EDTA blood samples were centrifuged at 3000 g for 10 min at 4˚C, and the plasma was quickly removed and stored at –20˚C until HPLC analyses. A 500 µl portion of plasma was adjusted to pH 2.5 with 150 µl of 1M potassium dihydrogen phosphate solution and 15 µl 50% phosphoric acid. Each sample was vortex mixed with 2.5 ml acetonitrile for 1 min and centrifuged at 3500 g for 10 min at 5-10 ˚C. The supernatant was evaporated to dryness at 35˚C using a stream of liquid nitrogen. The residue was reconstituted in 100 µl methanol and 50µl (sample volume) was injected onto an HPLC system to determine the concentration of EA.

High-performance Liquid Chromatographic (HPLC) Analysis

Analysis was performed on Shimadzu binary liquid chromatography model LC-10AVP, the system equipped with shimadzu SPD 10 A vp UV_VIS spectrophotometers (Shimadzu ,Kyoto, Japan) , The mobile phase, solvent A 1.5% acetic acid in water and solvent B 1.5% acetic acid in methanol was used under binary linear gradient with a flow rate of 1.0 ml/min. The wavelength was monitored at 360 nm for detection and quantification of ellagic acid EA (standared EA obtained from Sigma, USA), typical chromatogram for standard EA is shown in Fig. 2 , the retention of Ea in chromatogram is 6.10 min.
EA standard(50µg/ml) was solubilized in DMSO and serially diluted to prepared 400, 200, 100, 50, 25 and 12.5 ng/ml solutions. Control plasma was spiked with individual solutions and extracted as previously outlined. Each plasma sample was separately extracted (x 3) and each sample was injected in triplicate on the HPLC. Concentrations were determined from the peak area by using the equation for linear regression obtained from the calibration curve. The calibration curve was linear (R = 0.9998) over the concentration range from 400 to 12.5 ng/ml. as shown in fig 1. The recoveries of EA from human plasma were 101, 107, 104 and 112 % for the concentrations 400, 200, 100,and 50 ng/ml, respectively.
The method was linear over a range of 12.5 – 400 ng/ml of ellagic acid in plasmas.

Pharmacokinetics and Metabolism

Following oral administration of ellagic acid capsule 80 mg, the maximum peak plasma concentrations of EA occur within about 1 hours, the concentration of EA from extracted plasma samples, with different time interval from 0-12 h, were measured and tabulated in table 1.

To compare the rate and extent of absorption of EA solutions, the following pharmacokinetic variables were calculated for each volunteer using actual blood sampling times. The areas under the plasma concentration-time curves (AUC 0-12 hr) were calculated using the linear trapezoidal rule. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were obtained

directly from the plasma-concentration data.

The elimination rate constant was calculated by least-squares regression using the last points of each curve; the pharmacokinetics parameter for EA was summarized in table 2.

Table (1) Mean Concentration of Ellagic Acid (ng/ml) in Plasma of 20 Healthy Volunteers after Oral Dose of 80 mg Ellagic Acid Capsule.

Time hours									
no	0.0	0.3	1	2	3	5	7	9	12
1	0	172	320	190	170	121	90	50	12.5
2	0	179	311	188	162	117	80	44	Nd
3	0	182	341	192	171	123	88	34	Nd
4	0	191	299	**185**	172	131	93	36	15
5	0	182	309	173	168	120	81	52	Nd
6	0	162	317	205	164	95	72	62	16
7	0	175	295	196	172	132	92	66	Nd
8	0	159	362	219	178	134	98	42	Nd
9	0	194	314	222	162	127	77	50	Nd
10	0	177	309	172	178	125	82	41	16
11	0	182	312	195	162	121	90	43	20
12	0	166	279	201	157	110	71	36	Nd
13	0	180	289	207	155	126	82	42	Nd
14	0	178	370	219	165	116	99	54	Nd
15	0	166	361	195	169	112	82	46	Nd
16	0	194	352	186	142	124	75	40	Nd
17	0	179	338	176	159	132	65	36	18
18	0	181	369	182	165	124	73	38	17
19	0	192	295	209	160	136	70	45	Nd
20	0	187	272	202	150	124	62	41	Nd
Mean	0	178.9	320.7	204.35	164.05	122.5	81.10	44.9	-
±SD	0	7.945	15.035	9.2175	7.2025	5.125	3.055	1.245	-

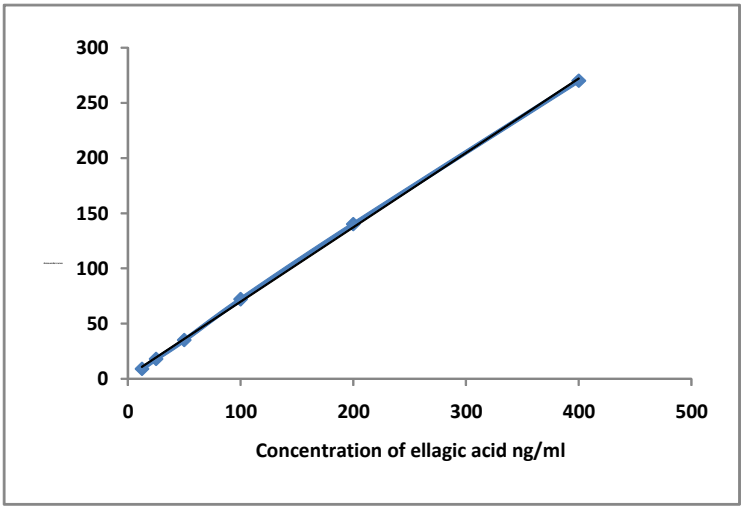

Fig. (1) Calibratnon Curve of Ellagic Acid

Results and Discussion

The optimum separation condition previously reported (Whitley.*et al* .2003) were modified by using fast liquid chromatographic column (50 mm length instead of 250 mm ,using high surface area ,3 µm particle size , the retention time Rt ellagic acid standard was 6.10 min Fig 2A, while the control plasma showed no corresponding peaks detected in the plasma sample (Fig. 2B) . However since EA was detected, control plasma was spiked with different concentrations of EA standard and processed according to the extraction procedure for quantification purposes. Fig 2 C shows the EA peak eluting at 6.06 min in spiked control plasma. EA was detected and quantified in plasma samples collected at 0.3h -12 h as shown in table 1. From this table, we clearly observed that most EA was not detected in plasma samples collected at 12h after oral administration. The concentration –time curve fig 3, Show that, the maximum absorption was achieved in 1 h, with maximum concentration of 320.7 ng/ml and half life was 6.12 h. this information is very important for estimation the dose and therapeutic concentration for various applications.

In conclusion, by combining an extraction procedure for plasma sample preparation and an HPLC-UV system, we have successfully obtained direct evidence of the absorption of EA in human plasma as data base for further studies related therapeutic effect of EA on cancer cells.

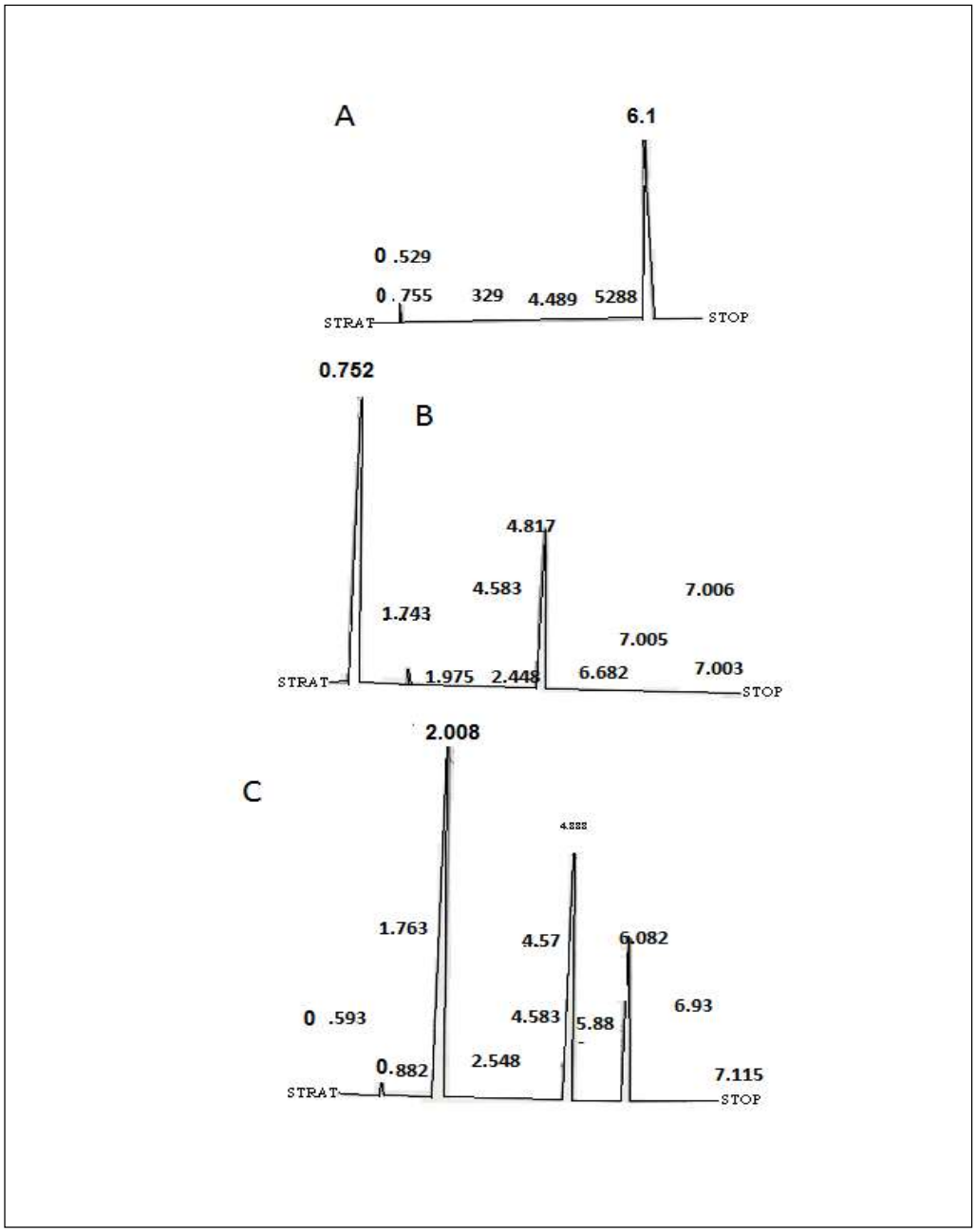

Fig. (2) Typical Chromatogram of (A) Standard Ellagic Acid Separated by Applied the Optimal Condition in the Text. (B) Blank Plasma Free Ellgic Acid , (C) Plasma from Patient after Oral Dose of 80mg of Ellagic Acid Rt=6.1 .

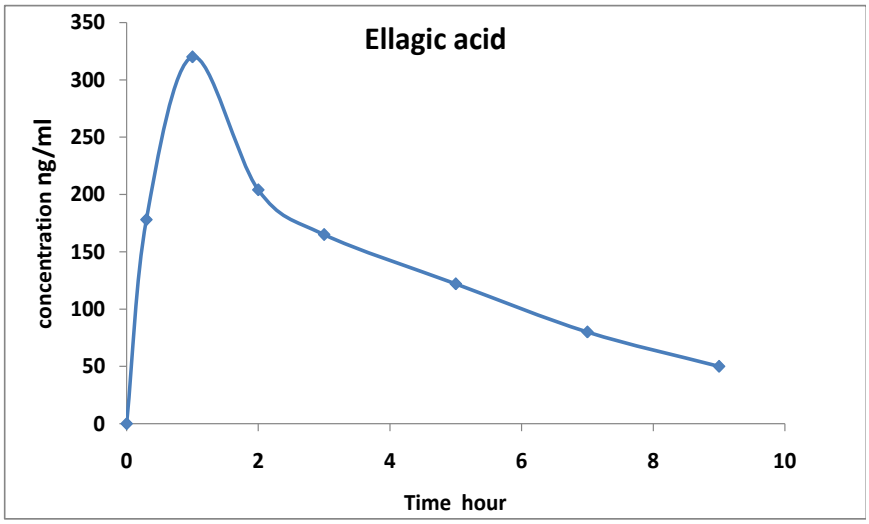

Fig. (3) Mean Plasma Concentration-time Curve of Ellagic After Oral Administration of 80 mg to 20 Healteers. Volunteer

Table (2) Mean value of Pharmacokinetic Parameters of Single Dose Administration of 80 mg Ellagic Acid to 20 Healthy Volunteers.

Subject No.	K_a	$K_{a\ 0.5t}$	$K_{elem.}$	K_{elem} 0.5t	Cmax	Tmax	AUC
Mean	7.24	0.156	0.092	6.20	320.7	1.0	1623.7
± SD	0.67	0.017	0.021	1.20	22.56	0.0	121.95

Ka: Time of Absorption,
Ka 0.5t: Half time of Absorption
K elem: Time of Elimination
Kelem 0.5t: Half Time of Elimination
C max: Maximum Concentration
T max: Maximum Time to Reach Maximum Concentration
AUC: Area Under Curve

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