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Bioavailability of Ellagic Acid After Single Dose Administration Using HPLC

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Abstract: Ellagic Acid (EA) is a complex planar molecule which demonstrates a variety of anticarcinogenic activities. Ellagic Acid acts as a scavenger to "bind" cancer-causing chemicals, making them inactive. It also has anti-bacterial, anti-viral properties. The concentration of EA was assessed in serum of twenty healthy volunteers over time interval from 0.15, 0.3, 1, 1.30, 2, 3, 6, 8 and 12 h. The serum concentration-time profile is presented after administration of 40 mg of ellagic acid to 20 healthy volunteers. Isocratic reversed phase liquid chromatographic method was developed for determination of low quantity of Ellagic Acid (EA) on reversed phase Lichrospher 100 RP-18, 5 µm (250 x 4.6 mm i.d) column and using methanol as mobile phase. The eluted EA was detected by UV set at 254 nm. Calibration curve for standard EA is linear with correlation coefficient of 0.9997 for detector response plotted against concentration range from 20-1000 ng/ml. The concentration of ellagic acid (C_{max}) was highest in blood one hour (T_{max}) after oral administration. It was 200.15±26.66 ng/ml., the mean serum elimination half-life was about 8.4±1.4 h. More than 50% of ellagic acid boud to plasma protein following oral administration, absorption of ionized ellagic acid occurs in stomach and intestine. Therefore, one capsule three times has drug bioavailability through all day.

Key words: Bioavailability of ellagic acid, HPLC separation

INTRODUCTION

Ellagic acid is a potent anti-oxidant, aphenolic compoundknown as apotent anti-carcinogenic, anti-mutagenic compound (Suzuki *et al*., 1990). Research shows that ellagic acid, which is an anti-carcinogenic, inhibits the growth of cancer cells (2). It also causes apoptosis, or normal cell death in those cancer cells (Loarca-Pina *et al*., 1998).

Clinical tests conducted at Hollings Cancer Institute at the Medical University of South Carolina (MUSC) has shown that ellagic acid, a naturally occurring plant phenol leads to G1 arrest of cancer cells (Daniel *et al*., 1991), thus inhibiting and stopping mitosis (cancer cell division). Cervical Cancer Cells-HPV (human papilloma virus) experienced apoptosis (normal cell death) when exposed to ellagic acid (Daniel *et al*., 1991). Nixon found that the ellagic acid causes apoptosis (normal cell death) of human cervical cancer cells and induces G1 inhibition of cancer cell division and prevents destruction of the P53 gene by cancer cells. P53 is regarded as a safeguard against mutagenic activity (cancer causing changes) in cervical cells (Nixon, 1999; 2000).

Tests reveal similar results for breast, cervical cancer, pancreas, esophageal, skin, colon and prostate cancer cells. European medical studies also demonstrate that Ellagic Acid is known to lower the incidence of birth defects, promote wound healing, reduce heart disease, and may reduce or reverse chemically induced liver fibrosis.

Mechanism of action: Most of the researchers found that the ellagic acid acts as a scavenger to "bind" cancer-causing chemicals, making them inactive. It inhibits the ability of other chemicals to cause mutations in bacteria. In addition, ellagic acid prevents binding of carcinogens to DNA and reduces the incidence of cancer in cultured human cells exposed to carcinogens. One method by which cancer affects DNA is through covalent bonding of the carcinogen to the DNA molecule. Ellagic acid inhibits mutagenesis and carcinogenesis by forming abducts with DNA, thus masking binding sites to be occupied by the mutagen or carcinogen (Teel and Castonguay, 1992 and Smith *et al*., 2001; Narayanan and Re, 2001).

Ellagic acid is active in antimutagenesis assays, and has been shown to inhibit chemically induced cancer in the lung, liver, skin and esophagus of rodents and TPA-induced tumor promotion in mouse skin (Whitley *et al*., 2003).

Ellagic acid elicits a dose-dependent bactericidal effect in H. pylori cultures, the bacteria thought primarily responsible for the development of gastric ulcers (Chung).

Ellagic acid significantly reduces the elevated levels of enzymes, lipid peroxide and liver hydroxy proline and rectifies liver pathology in laboratory animal hepatotoxicity induced by carbon tetrachloride (Thresiamma).

Ellagic acid inhibits lipid peroxidation necrosis of skin flaps, enhancing preservation of grafting procedures (Ashoori, 1994).

Ellagic acid treatment of preweanling mice before an injection of B(a)P diol-epoxide caused a 44-75%

inhibition in the number of diol-epoxide-induced lung tumors (Chang).

Ellagic acid inhibits N-nitrosomethylbenzylamine (NMBA) tumorigenesis in the esophagus of F-344 rats. Ellagic acid inhibited the development of both preneoplastic and neoplastic lesions by 25-50% (Daniel and Stoner). Tanaka *et al.* (1993) found that the inhibition of 4-nitroqinoline-1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenols.

MATERIALS AND METHODS

Chemicals: Methanol HPLC grade were obtained from Merck U.S.A, Ellagic acid certified standard and purified was obtained from Sigma; Deionized water was filtered by Milli-RO plus together with a Milli-Q system from Millipore (Bedford, MA. USA)

Column: Chromatographic separation was carried out on a LiChrospher 100 RP-18, 5 µm particle size (250 x 4 mm i.d) column obtained from Suplico Co., (Darmstadt, USA).

Chromatographic conditions: The liquid chromatography consisted of two LC-8A pumps, a Sil-6AV auto injector and SCL-8A system controller 10AVP UV\Visible detector and data processor which have the ability to draw the chromatograms and calculate the peak area, peak height and concentrations simultaneously, all purchased from Shimadzu, (Kyoto, Japan). The column used was Suplico C-18 DB (250 X 4.6 mm I.d.). The mobile phase was pure methanol. The flow-rate was 1 ml/min and the temperature 25°C, the injection volume was 20 µl and the detector was set at 254 nm.

Standard solution: A stock solution of EA was prepared by dissolving 1 mg of EA accurately weigh in 10 ml of pyridine. This solution was further diluted in mobile phase to obtained appropriate concentrations for calibration between 20-1000 ng/ml.

Extraction of serum samples: The blood samples (3 ml) were collected from 20 healthy volunteers (Table 1), age 20-40 years, body weigh 55-84 Kg, their height 160-174 cm, after oral administration of 40 mg EA capsule, the blood was collected in different times between 0.15-12 h, in single randomized studies. Serum were obtained from blood samples and centrifuged at 900 g. Then 20 µl of aliquots subjected to HPLC analysis.

Linearity of the chromatographic method: The typical chromatogram obtained for a standard of EA is shown in Fig. 1. The retention time of ellagic acid in chromatogram is about 6 min. The instrument precision,

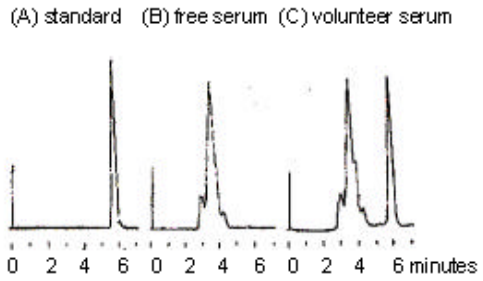

Fig 1: Chromatograms of (A) pure ellagic acid standard, (B) blank serum and (C) serum from a volunteer after the administration of 40 mg capsule of ellagic acid

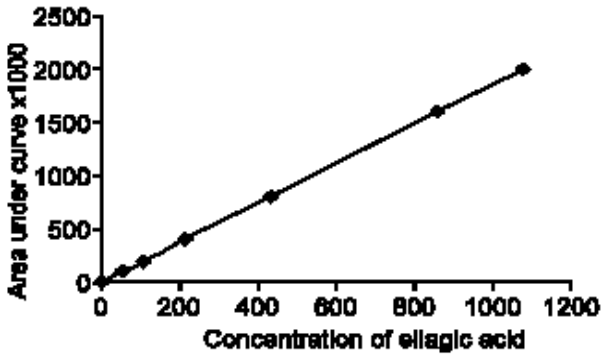

Fig. 2: Calibration curve of Ellagic acid

Table 1: Characteristics of the volunteers

Subject	Sex	Age\years	Height \cm	Weight\kg
1	M	23	170	68
2	M	24	165	65
3	M	26	173	63
4	M	29	166	70
5	M	20	174	80
6	M	35	172	78
7	M	32	171	74
8	M	27	167	75
9	M	33	169	73
10	M	29	173	84
11	M	30	168	72
12	M	40	174	69
13	M	24	160	58
14	M	23	164	57
15	M	24	166	60
16	M	26	168	55
17	F	29	160	59
18	F	30	161	60
19	F	32	165	58
20	F	34	165	60
Mean		28.5	167.5	66.90
±SD		4.94	4.21	8.59

determined by eighty successive injections of the standard preparation, exhibited a maximum RSD (t_R) of 0.2%. The column efficiency was more than 15000 theoretical plates. The tailing factor was not more than 1.2 at 5% peak height. An acceptable correlation

Table 2: Mean concentration of Ellagic Acid (ng\ml) with time in sera of 20 healthy volunteers after orall dose of 40 mg ellagic acid capsule

	Time-Hours								
No.	0	0.15	0.30	1.00	1.50	2.00	3.00	6.00	8.00	12.00
1	0	35.34	128.00	244.11	182.27	146.66	141.06	123.97	98.44	50.34
2	0	44.00	123.33	173.71	164.13	140.69	133.16	116.89	103.32	83.42
3	0	38.12	140.92	157.34	156.70	145.79	138.17	131.94	110.38	55.27
4	0	40.20	169.88	183.39	172.49	150.06	140.64	127.37	109.58	49.64
5	0	24.65	125.76	193.67	150.45	130.66	100.54	80.45	65.47	35.45
6	0	28.31	133.98	210.44	140.66	110.24	90.66	82.16	60.22	38.10
7	0	31.22	138.32	226.78	179.56	139.56	111.23	98.44	74.63	34.66
8	0	42.46	141.56	199.68	160.22	124.88	116.43	93.56	62.64	32.63
9	0	37.00	122.65	188.66	145.56	123.67	98.98	88.55	60.47	20.42
10	0	43.86	144.36	230.78	166.40	140.32	120.65	100.76	80.66	41.00
11	0	45.66	155.67	242.56	180.88	132.44	98.78	88.56	68.98	27.88
12	0	45.34	153.88	188.66	140.34	123.54	103.65	89.54	72.67	22.90
13	0	47.44	144.56	168.76	132.76	113.68	83.56	98.41	79.67	23.10
14	0	48.00	156.54	167.44	140.23	123.86	81.24	69.44	55.66	20.00
15	0	42.20	148.67	177.48	141.96	120.15	100.32	85.00	65.00	19.57
16	0	36.34	139.22	189.04	153.78	119.45	108.61	91.47	80.21	17.77
17	0	38.68	147.00	197.78	140.50	118.56	92.52	80.21	70.45	18.44
18	0	44.50	159.55	220.66	178.88	150.24	115.84	100.42	91.22	27.15
19	0	33.12	138.70	242.48	186.45	144.78	128.50	116.74	98.67	22.64
20	0	44.00	145.42	200.23	160.57	131.87	121.25	111.00	93.10	28.00
Mean	0	39.52	142.89	200.185	156.79	132.11	112.04	99.144	80.072	33.419
±SD	0	6.490	12.529	26.667	16.571	12.332	17.650	17.515	17.360	12.718

Table 3: Mean values of the pharmacokinetic parameters following oral administration of the Ellagic Acid 40 mg capsule

No.	Ka.	Ka 0.5t	Kelem.	Kelem 0.5t	Cmax.	Tmax.	AUC
1	5.149	0.135	0.078	8.908	244.11	1	1366.763
2	5.432	0.127	0.072	9.625	173.71	1	1254.342
3	5.527	0.125	0.068	10.191	157.34	1	1102.352
4	6.200	0.111	0.073	9.493	183.39	1	954.637
5	6.519	0.106	0.087	5.5.19	193.67	1	992.793
6	6.219	0.114	0.095	7.319	210.44	1	896.355
7	4.460	0.155	0.065	10.662	226.78	1	878.674
8	5.736	0.121	0.081	8.556	199.68	1	968.442
9	5.238	0.132	0.083	8.349	188.66	1	1286.325
10	4.766	0.145	0.122	5.697	230.78	1	1186.575
11	5.226	0.133	0.082	8.451	242.56	1	976.634
12	6.645	0.104	0.074	9.365	188.66	1	886.534
13	4.679	0.148	0.069	10.043	168.76	1	1312.126
14	4.754	0.146	0.119	5.824	167.44	1	1110.682
15	5.038	0.138	0.128	5.400	177.48	1	843.054
16	4.754	0.146	0.063	11.000	189.04	1	976.412
17	4.762	0.145	0.076	9.118	197.78	1	866.347
18	6.348	0.109	0.132	5.250	220.66	1	838.832
19	5.422	0.128	0.070	9.900	242.48	1	1010.538
20	4.783	0.145	0.075	9.219	200.23	1	1127.778
Mean	5.383	0.131	0.085	8.394	200.185	1	1041.810
±SD	0.681	0.016	0.022	1.884	26.667	0	166.803

Ka: Time of absorption | Ka 0.5t: Half time of absorption | Kelem: Time of Elimination
Kelem 0.5t : Half Time of Elimination | Cmax: Maximum Concentration | Tmax: Maximum Time
AUC: Area Under Curve

coefficient of 0.9997 was obtained for the detector response plotted against ellagic acid concentration ranging between 20-1000 ng/ml as shown in Fig 2. The detection limit was 15 ng/ml.

RESULTS AND DISCUSSION

A fast and accurate isocratic reversed phase method was developed for separation of EA with maximum RSD for retention time was 0.20%. The procedure was free from endogenous interference from EA spiked plasma as shown in typical chromatogram Fig. 2. An acceptable correlation coefficient of 0.997 was obtained for standard calibration of EA in the range between 20-1000 ng/ml.

Ellagic Acid (EA) is absorbed rapidly from the gastrointestinal tract. Fifteen minutes following oral administration of EA. The 30-50 ng/ml was absorbed

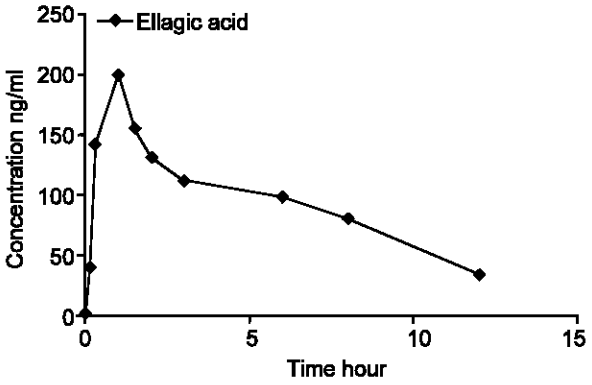

Fig. 3: Mean plasma concentration-time curve of ellagic acid after oral administrated 40 mg capsule to twenty healthy volunteers

and available in the blood serum. EA is about 50% bound to plasma protein with half-life of 8.4 h.

Blood sample was collected from each subject pre-dose and at 0.15, 0.30, 1.0, 1.30, 2, 4, 6, 8 and 12 h post-dose. The plasma peak concentration time of EA were measured and tabulated in Table 2 and Fig. 3. The level of ellagic acid in blood was low and probably, almost the entire absorbed dose was excreted in urine (Teel and Martin, 1988). The results of free ellagic acid in serum was given in Table 2, these results showed that 50 % of total ellagic acid as labeled with blood protein (Fig. 3). The ellagic acid and its metabolite are absorbed from the gastrointestinal tract. Following oral administration, absorption of ionized ellagic acid occurs in stomach and intestine, after absorption, the ellagic acid is rapidly converted to labeled protein ellagic acid during the first 30 min following oral administration. Ellagic acid is the predominant from of drug in serum, the percentage of ellagic acid is about 50-60% bound to serum protein with half life of 8.4±1.8 h. The results of this study is support our hypothesis, that the deproteinized serum ellagic acid was about 50% as free ellagic acid and the remaining of ellagic acid bound to serum protein. The pharmacokinetics parameters were calculated from the area under the curve AUC, the results presented in Table 3. The maximum serum peak concentration of EA was 200.2±26.7ng/ml reached at 1 h.

Conclusion: The pharmacokinetic profile indicate that ellagic acid has poor absorption and rapid elimination after oral administration of pomegranate leaf extract and part of it was absorbed directly from stomach.

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