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Research Article

MAPPING PHARMACOKINETIC AND TISSUE DISTRIBUTION PROFILE OF [6]-SHOGAOL FROM GINGER OLEORESIN

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ABSTRACT

In present study *in vivo* fate of [6]-shogaol (SGL) was explored upon oral administration of Ginger Oleoresin (GO) by determining its level in plasma with special emphasis on its distribution pattern in various tissues by rapid and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method. The SGL showed major distribution along the gastrointestinal tract, liver, heart, lung with traceable amount in brain. The highest concentration of SGL among all the tissues was found to be less than 0.05% of administered dose of SGL, confirming it's poor bioavailability. The SGL exhibited diminutive elimination half life and mean residence time in liver. Further higher elimination half life and mean residence time of SGL in small intestine (SI) suggests that SGL follows enterohepatic circulation pathway. The study findings will be helpful in future investigation on efficacy of SGL and its metabolites in correlation with its bioavailability.

Keywords: Ginger Oleoresin, [6]-shogaol, Pharmacokinetics, Tissue Distribution, RP-HPLC.

INTRODUCTION

Oleoresin of ginger (GO) is one of the widely used herbal remedies for treatment of several ailments such as emesis, inflammation, cough etc. since traditions [1,2]. GO is a complex mixture of non-volatile pungent components extracted from *Zingiber officinale* Roscoe, comprising homologous series of phenolic ketones such as gingerols and shogaols [3]. [6]-shogaol (SGL) (Fig.1), one of the components of GO is known to be responsible for antipyretic, antimicrobial, anti-inflammatory, cardiotonic, analgesic and antitussive activity [1,2,4,5,6]. Several *in vitro* studies have demonstrated role of SGL in suppressing the carcinogenesis of ovary [7], lungs [8], colon [9], gastrointestinal (GI) tract [10] and neuroblastoma [1]. The SGL metabolites are also known to posses anticancer activity [11].

A medicinal product exerts its therapeutic activity once it achieves the therapeutic level at the site of action [12]. Paramount step in the development of a drug for any disease treatment is understanding of its distribution, metabolism and excretion upon oral administration to preclinical species. Although SGL has been reported to posses wide array of therapeutic effects [6-10], it exhibits poor bioavailability on account of its minimal amount in systemic circulation [13-15].

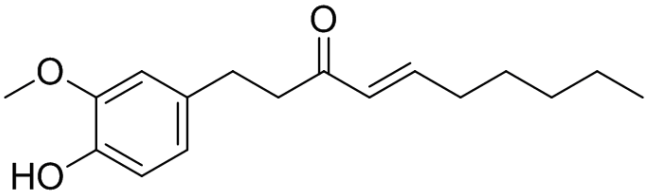

Fig. 1: Chemical Structure of [6]-shogaol [$C_{17}H_{24}O_3$]

Recent studies have shown that, SGL undergoes biotransformation by glucorunic acid and sulfate conjugation and further, excretes through feces via bile which contributes to poor bioavailability of unchanged SGL [11,13-17]. Although SGL has been extensively studied for its metabolism and excretion, its tissue distribution pattern has not been explored yet. Therefore, in order to ascertain the therapeutic efficiency of SGL, it is necessary to study the *in vivo* fate of SGL.

Hence, the objective of the present study was to explore the fate of SGL in various tissues upon oral administration of GO to Wistar rats. To the best of our knowledge, this is the first attempt made to evaluate tissue distribution profile of SGL upon oral administration of GO.

MATERIALS AND METHODS

Chemicals

GO (Ginger Oleoresin) was purchased from Nisarg Biotech Satara, Maharashtra, India and was in house standardized for the content of SGL (2.03% w/w) [15]. The reference standard of SGL (>96% purity w/w) and IS (internal standard) β-estrodiol (>97% purity w/w) was procured from Natural Remedies Pvt. Ltd. Bangalore, Karnataka, India. Vacutainers coated with K2EDTA were purchased from BD, Becton, Dickinson and Company India. All other reagents and chemicals used in this study were of analytical grade and procured from Merck Specialties Private Limited, Mumbai, India.

Animals

Wistar rats weighing between 190 to 210 g were purchased from National Institute of Bioscience, Pune, India. The animals were housed at an ambient temperature 25±2°C and relative humidity 50±2% and light and dark cycle (12 hr light/dark). The animals had access to pellet diet (Chakan oil mills, Pune, Maharashtra, India) and water ad libitum.

Research protocol approval

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India (Approved protocol no- CPCSEA/52/2010; institutional approval no- CPCSEA/1999/100).

Preparation of dosing solution

Dosing solutions were prepared by dissolving GO (300 mg/kg) [14] in olive oil [18]. Freshly prepared dosing solution was administered orally via gavage.

Animal Studies

Pharmacokinetic study

Twelve Wistar rats were divided in two groups (n=6) and fasted overnight (12 hr). A single dose of GO (300 mg/kg) was administered orally. Blood samples (Approximatly 0.8 mL) were collected by retroorbital plexus into vaccutainers coated with K2EDTA at 5, 30, 60 and 120 min from the first group and from the other group it was collected at 15, 45, 90 and 180 min after administration of GO. Collected blood samples were centrifuged at 4000 *rpm* for 10 min and plasma was harvested within 30 min. All plasma samples were frozen and stored at -70°C until further analysis.

Tissue distribution study

Fourty eight Wistar rats were devided in eight groups (n=6), fasted overnight (12 hr) and administered a single oral dose of GO (300 mg/kg). Each group was sacrificed at specific time interval of 15, 30, 60, 90, 120, 180, 240 and 360 min postdose, respectively. Tissues viz. brain, heart, lung, liver, spleen, kidney, stomach and SI were collected from each group. The tissues were rinsed with water, blotted dry and frozen at -70^{0}C and subjected to analysis.

Sample preparation

Plasma

To the mixture of rat plasma (450 µL) and internal standard (50 µL), ethyl acetate (1.5 mL) was added and vortexed for 2 min followed by centrifugation at 12,000 rpm for 10 min at 4^{o}C. After centrifugation the supernatant layer was evaporated to dryness at 40^{o}C under a gentle stream of nitrogen. Finally, the residue was reconstituted using 200 µL of methanol and subjected to HPLC analysis.

Tissue

Each tissue was homogenized in 0.9% saline solution (500 mg/mL). To the mixture of tissue homogenate sample (450 µL) and internal standard (50 µL), diethyl ether (1.5 mL) was added and mixed for 30 s. After centrifugation (4000 rpm for 15 min at 4^{o}C), the organic layer was transferred to a clean glass tube. The remnant tissue homogenate layer was re-extracted using diethyl ether (1.5 mL). The above two extracts were combined and dried under a gentle stream of nitrogen. The residue was reconstituted in 100 µL of methanol and subjected to HPLC analysis.

HPLC conditions

The HPLC system (Jasco corporation, Tokyo, Japan) consisting of a Pump (model Jasco PU- 2080 Plus) with UV/VIS detector (Jasco, UV 2075) and Jasco Borwin version 1.5, LC-Net II/ADC system software was used for the analysis. Liquid chromatographic separations were performed on a BDS Hypersil C18 analytical column with dimensions 250 × 4.6 mm, particle size 5 µ (Thermo Scientific, Waltham, USA). The mobile phase used was acetonitrile and (0.1%) ortho-phospohoric acid (70:30 v/v) at a flow-rate of 1.0 mL/min. The chromatograms were monitored at 281 nm.

Preparation of standard solution, calibration standard and quality control sample

Stock solution of SGL was prepared in methanol. Calibration standards of SGL for plasma were prepared in the concentration range of 0.033–3.333 µg/mL; for stomach, SI and liver homogenate 0.267-26.667 µg/mL and 0.133-8.000 µg/mL for lungs, heart, kidney, spleen and brain homogenates. Quality control (QC) samples were prepared similarly.

Method validation

The proposed method was validated as per the US Food and Drug Administration guidelines for bioanalytical method validation [19].

Statistical analysis

Statistically data was analyzed by Graph Pad Prism 4.0 and subjected to pharmacokinetic analysis using WinNonlin 5.2 software. All the values were expressed as mean±SD. Measurement data were analyzed using a one-way analysis of variances (ANOVA) followed by *post hoc* Bonferroni test. The result of $P<0.05$ was considered statistically significant.

RESULT AND DISCUSSION

In the present study rapid and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method was developed to identify SGL in plasma and selected tissues after oral administration of GO in Wistar rats. Resolution of SGL was achieved using mobile phase acetonitrile: (0.1%) ortho-phosphosphoric acid in a ratio of 70:30 v/v and the SGL was detected at retention time of 10.30 min. The calibration curve of SGL was constructed for each biological sample and found to be linear over a certain range in all biological samples with a correlation coefficient (r) larger than 0.996 (Table 1).

Table 1: The linear regression equations for calibration curves of SGL in rat plasma and tissue samples.

Sample	Regression Equation	Correlation Coefficient (r^2)	Linearity range (µg/g)
Plasma	y = 0.0019x + 0.0259	0.9997	0.033-3.333[a]
Stomach	y = 0.0019x - 0.022	0.9989	0.267-26.667
Small Intestine	y = 0.0021x - 0.0059	0.9989	0.267-26.667
Liver	y = 0.0005x + 0.0894	0.9995	0.267-26.667
Lungs	y = 0.0022x + 0.0051	0.9969	0.133-8.000
Heart	y = 0.0021x + 0.0586	0.9997	0.133-8.000
Kidney	y = 0.0015x + 0.0618	0.9995	0.133-8.000
Spleen	y = 0.0018x + 0.0325	0.9986	0.133-8.000
Brain	y = 0.0013x + 0.0349	0.9979	0.133-8.000

[a]Linearity Range for Plasma: µg/mL

The LLOQ (Lower Limit of Quantitation) was found to be 0.033 µg/mL for plasma, 0.133 µg/g for lungs, heart, kidney, spleen, brain and 0.267 µg/g for stomach, liver and small intestine (SI), respectively. Further, precision and accuracy study results showed that for each low, middle and high QC (Quality Control) samples, the relative standard deviation (%RSD) was lower than or equal to 20% (Table 2). The extraction recoveries (86.000–90.000%) for each QC sample were in acceptable range for each biological matrix (Table 2). The stock solution of SGL was found to be stable for 6.00 hrs at room temperature whereas it was found to be stable up to 21 days at 2-8^{o}C. The freeze-thaw stability and bench top stability study results were also within range of ± 10% of nominal value.

Non-compartmental analysis was performed to analyze the pharmacokinetic parameters of SGL [Table 3]. SGL was detected in plasma at 5 min after oral administration of GO and was found up to 180 min post dose (Fig.2). Maximum concentration (C_{max}:1.502±0.111 µg/mL) of SGL was achieved rapidly at 30.00±0.000 min (T_{max}) post dose however elimination of SGL from plasma was also rapid as reflected by low elimination half life ($T_{1/2z}$:26.00±1.280 min) and low mean residence time (MRT_{0-t}:58.760±1.564 min). The study findings are in line with the previous reports stating poor bioavailability of SGL [13-15] as less than 0.05% of administered dose of SGL was detected in plasma.

SGL was detected at 15 min in all the selected tissues viz stomach, liver, heart, SI, spleen, lungs and brain. However in kidney it was detected at 30 min (Table 4). The maximum concentration of unchanged SGL in stomach (15.608±0.567 µg/g), liver (16.546±1.342 µg/g), SI (13.676±1.180 µg/g), heart (5.899±0.518 µg/g) and lungs (8.014±0.310 µg/g) was observed to be significantly higher than plasma (1.502±0.111 µg/mL) ($P<0.01$). However maximum concentration of SGL was non-significantly higher in kidney (1.671±0.221 µg/g), on other hand it was non-significantly lower in spleen (1.243±0.090 µg/g) as well as in brain (0.637±0.027 µg/g) compared to plasma (Fig.3A). The observed elevated levels of SGL in liver, stomach and SI and higher area under curve (AUC_{0-t}) values thereof (Fig.3B) in comparison to that of other tissues confirm the liver, stomach and SI to be the main site of absorption, distribution and metabolism of SGL as proposed previously [15]. Highest level of SGL was achieved at 15 min (T_{max}) post dose in stomach, 30 min in liver, heart, SI, spleen and lungs whereas it was achieved at 60 min in kidney and brain.

Table 2: Intra-day and inter-day variability, Staibility and recovery of SGL in rat plasma and liver tissue homogenate (n=6).

Validation Parameter	QC Concentration (µg/mL)	Plasma Precision (%RSD)	Accuracy (% RE)	QC Concentration (µg/mL)	Liver Precision (%RSD)	Accuracy (% RE)
Intra day	0.067	3.766	-0.034	0.667	2.970	-0.039
	1.333	2.978	-0.038	5.333	0.884	0.041
	3.333	1.309	-0.051	13.333	0.346	-0.012
Inter Day	0.067	4.406	-0.003	0.667	4.526	-0.039
	1.333	17.040	-0.006	5.333	4.375	0.041
	3.333	1.580	-0.056	13.333	4.111	-0.012
Freeze Thaw Stability	0.067	4.697	0.080	0.667	4.750	-0.109
	3.333	2.410	-0.002	13.333	3.350	-0.030
Bench top Stability	0.067	10.308	0.050	0.667	4.760	-0.092
	3.333	1.952	-0.010	13.333	3.420	-0.015
% Recovery*	0.067	89.654		0.667	89.817	
	1.333	86.401		5.333	86.877	
	3.333	89.339		13.333	86.976	

* Recovery was expressed in %

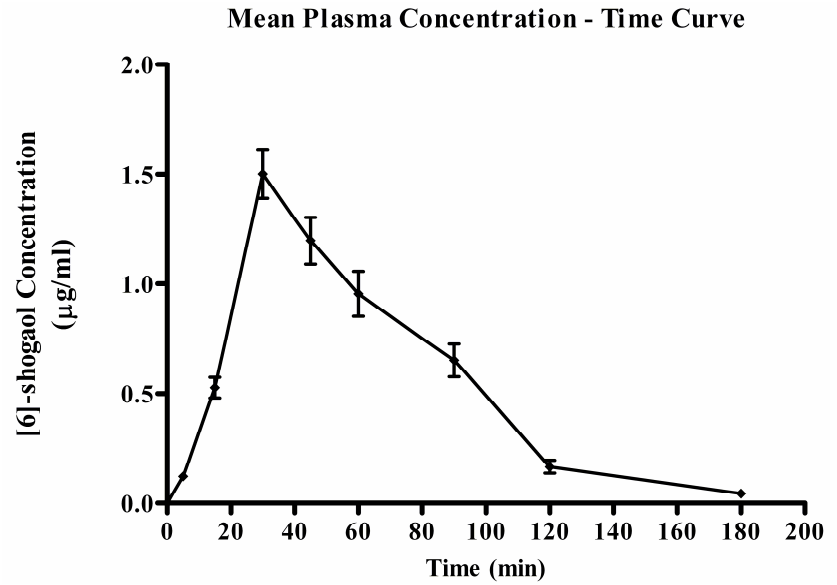

Fig. 2: Mean Plasma Concentration-Time profile of SGL after oral administration of GO (300 mg/kg) (Mean±SD) (n=6)

Table 3: Non-compartmental pharmacokinetic parameters of SGL in rat plasma after oral administration

Parameter	Value	Parameter	Value	Parameter	Value
C_{max} (µg/mL)	1.502±0.111	$AUMC_{0-t}$(µg min/mL)	5758.000±716.300	$T_{1/2z}$ (min)	26.00±1.280
T_{max} (min)	30.00±0.000	$AUMC_{0-\infty}$(µg min/mL)	6996.000±685.100	$V_{z/F}$ (mL/kg)	744.700±82.910
AUC_{0-t}(µg min/mL)	97.800±9.886	MRT_{0-t} (min)	58.760±1.564	$CL_{z/F}$ (mL/min/kg)	12.710±1.723
$AUC_{0-\infty}$(µg min/mL)	100.100±9.203	$MRT_{0-\infty}$ (min)	69.990±5.041		

Table 4: Distribution of SGL in tissues after oral administration of Ginger Oleoresin (300 mg/kg) in rats (n=6)

Time (Min)	Stomach (µg/g)	Liver (µg/g)	Heart (µg/g)	Kidney (µg/g)	SI (µg/g)	Spleen (µg/g)	Lungs (µg/g)	Brain (µg/g)
15	15.608±0.567	13.722±1.114	1.760±0.412	BD	2.338±0.497	0.776±0.066	0.456±0.015	0.133±0.005
30	6.766±0.350	16.546±1.342	5.899±0.518	0.723±0.191	13.676±1.180	1.243±0.090	8.014±0.310	0.296±0.011
60	2.086±0.173	5.073±1.156	4.398±0.189	0.987±0.106	7.732±0.582	0.862±0.057	2.521±0.185	0.637±0.027
90	1.039±0.090	0.486±0.454	3.409±0.226	1.671±0.221	4.634±0.389	0.738±0.066	0.815±0.094	0.090±0.070
120	0.725±0.031	BD	1.536±0.321	1.111±0.071	2.095±0.380	0.426±0.057	0.434±0.054	BD
180	0.431±0.026	BD	0.235±0.120	0.396±0.060	0.915±0.071	0.168±0.090	0.130±0.065	BD
240	BD	BD	BD	0.173±0.027	0.368±0.023	0.139±0.108	BD	BD
360	BD	BD	BD	BD	BD	BD	BD	BD

*BD: Below Detection Level

SGL exhibited variable elimination pattern from the selected tissues wherein the rate of elimination of SGL from liver was higher than that of other tissues reflected by lower elimination half life (17.43±2.127 min) compared to other tissues (Fig.3C). Further, SGL exhibited significantly (P<0.01) elevated elimination half life of 48.41±4.434 min, 45.16±3.678 min and 71.02±16.010 min from SI, kidney and spleen, respectively compared to that from liver. Also SGL showed lower mean residence time of 33.24±1.406 min in liver than that of 72.91±1.797 min in SI, 99.63±4.533 min in kidney and 88.38±15.560 min in spleen, respectively (P<0.01) (Fig.3D). The observed lower values of elimination half life and mean residence time of SGL in liver, confirms it to be the major site of metabolism and excretion of SGL as proposed previously [11], [15-17]. The elimination half life and mean residence time of SGL in SI reflects retention of SGL in SI. These observations suggest that SGL might have metabolized through enterohepatic circulation. This subsequently gets eliminated through feces [11], [15-17]. Elevated mean residence time and low AUC_{0-t} values of SGL in kidney and spleen confirms partial excretion of SGL from kidney as proposed previously [15]. Spleen is known to act as site of storage and destruction for RBCs [20]. It may be the cause of elevated elimination half life of SGL in spleen, subsequently leading to the retention of SGL in spleen.

Several *in vitro* studies state variable effective concentration of SGL to exert desired effects. For instance, 27 µg/mL or 100 µM of pure SGL inhibits the AKT/mTOR pathway in human non-small cell lung cancer A549 cells *in vitro*[8]. In the present study administered dose of 300 mg/kg of GO, comprising 2.03% w/w of SGL i.e. around 6.00 mg/kg of SGL achieves less than 0.05% of unchanged SGL in plasma as well as all the selected tissues. The maximum concentration of unchanged SGL found in lungs was found to be 8.014±0.310 µg/g which is much less than the required minimum effective concentration as reported for previous studies[8]. On the other hand there are number of *in vivo* studies reporting efficacy of pure SGL, for example, 6.2 mg/kg of pure SGL has been reported to reduce edematous swelling of the knees in inflammatory rats[5]. Further 2.0 mg/kg of pure SGL is reported to increase intestinal blood flow in Sprague–Dawley rats [21]. However SGL undergoes extensive metabolism due to which minimal amount of unchanged SGL reaches to systemic circulation [11], [13-17]. Therefore, it can be hypothesized that the SGL metabolites produced might also contribute to the reported therapeutic activity of SGL. Hence, there is need of comparative evaluation of efficacy of unchanged SGL and its metabolites. Further the *in vitro-in vivo* correlation of SGL and its metabolites needs to be ascertained.

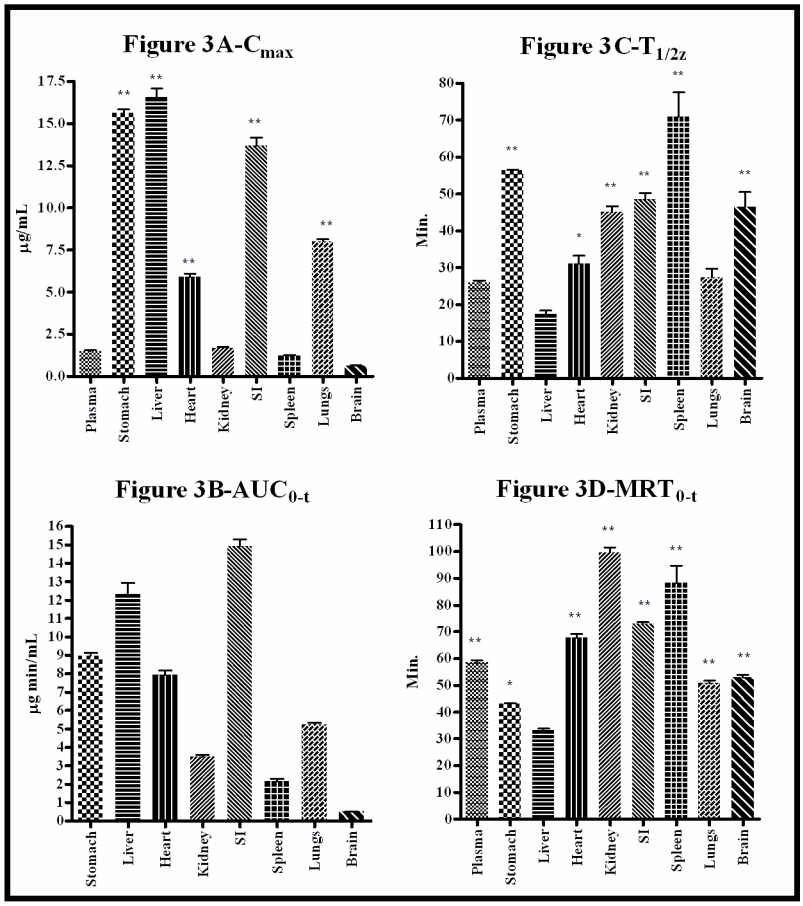

Fig. 3: Comparative tissue distribution profile of SGL.

Values are expressed as Mean±SD, (n=6). Data was analyzed by One-way ANOVA followed by *post hoc* Bonferroni test. P<0.05 considered as significant. **P<0.01 compared to plasma maximum concentration (Fig.3A-C_{max}), * P<0.05, **P<0.01 compared to liver elimination half life (Fig.3C-$T_{1/2z}$), * P<0.05, **P<0.01 compared to liver mean residence time (Fig.3D-MRT_{0-t}).

In summary, the present study contributes to understand the pharmacokinetic and tissue distribution profile of SGL upon oral administration of GO. The SGL has been found to be widely distributed in GI tract, liver, lungs and heart with traceable concentrations found in spleen, kidney and brain. However even in tissues with extensive distribution of SGL, maximum concentration of SGL was found to be less than 0.05% of administered dose confirming poor bioavailability of SGL. The SGL exhibits diminutive elimination half life and mean residence time in liver. Further higher elimination half life and mean residence time of SGL in SI suggest that SGL follows enterohepatic circulation pathway. The study suggests need of intensive investigation on efficacy of pure SGL and its metabolites in correlation with its bioavailability.

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