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Original Article

Pharmacokinetics of gallic acid from the topical ointments of standardized extracts of *Chrysophyllum cainito* and *Mimusops elengi* in rats

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ABSTRACT

Introduction: The wound healing activity of leaves of *Chrysophyllum cainito* and flowers of *Mimusops elengi* has been reported through our previous studies. Therefore, the aim of this study was to evaluate pharmacokinetics of gallic acid from the ethanolic extracts of these plants and to support the findings of the preclinical study. **Methods:** A simple HPLC method was developed for detection of gallic acid in rat plasma post topical application of the ointments containing 20% ethanolic extracts of both the plants and validated as per USFDA guidelines. Clonidine hydrochloride was used as an internal standard (IS). Chromatographic separation of gallic acid and IS was achieved on Cosmosil C_{18}-column using mobile phase [10 mM KH_2PO_4 in water: acetonitrile: orthophosphoric acid (95: 5: 0.05, v/v/v)] delivered at a flow rate of 1.0 mL/min. The ultraviolet detection wavelength was set at 215 nm. **Results:** The calibration curve was linear over a concentration range of 0.05-5.0 µg/mL. The method was found to be accurate, precise and stable for gallic acid from spiked plasma at QC levels. The average method recoveries for gallic acid were over 85% and extraction recoveries between 78 and 87%. **Conclusion:** The HPLC method was applied successfully to the pharmacokinetic study of gallic acid. The detection of gallic acid in rat plasma post topical application of ethanolic extracts of both the plants proves its absorption which probably facilitates the biochemical changes and thus exhibits wound healing activity of the plant extracts.

Key words: *Chrysophyllum cainito*, *Mimusops elengi*, Ethanolic extract, Gallic acid, HPLC, Pharmacokinetics.

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INTRODUCTION

Gallic acid (3, 4, 5-trihydroxybenzoic acid), a phenolic compound belonging to the category of phenolic acids (hydroxybenzoic acids), is commonly found in plants.[1] It has been reported to possess therapeutic activities including antioxidant, antiangiogenic, anticancer, wound healing, antidiabetic, cardioprotective, anthelmintic, antiviral, antimalarial, diuretic, etc.[1-3]

Chrysophyllum cainito L. and *Mimusops elengi* L. are evergreen trees belonging to the family Sapotaceae. Fruits and leaves of *Chrysophyllum cainito* have been traditionally used in the treatment of various ailments.[4] *Mimusops elengi* has a long history in the traditional system of medicine and different parts of this plant have been reported for their medicinal value.[5] The leaves of *Chrysophyllum cainito* have been reported to possess the phytochemical constituents lupeol acetate, α-amyrin acetate, gallic acid, ursolic acid, β-sitosterol, lupeol etc.[6-7] Flowers of *Mimusops elengi* possess arbutin, gallic acid, quinic acid, epicatechin, myricetin, rutin, ursolic acid, β-sitosterol and lupeol.[8-10] As reported in our recently published studies, the ethanolic extracts of leaves of *Chrysophyllum cainito* and flowers of *Mimusops elengi* were standardized in terms of content of total phenolics and gallic acid. Furthermore, the traditional claim on their wound healing potential was supported using excision wound rat model.[11-12]

Therefore, in the present research work, the pharmacokinetic profile of the bioactive marker gallic acid was studied post topical application of the ointment containing ethanolic extract of *Chrysophyllum cainito* and *Mimusops elengi* in male Wistar rats using a validated HPLC method. To date, several studies on pharmacokinetics of gallic acid from different biological matrices using various experimental models have been reported.[13-17] However, the present research work is the first attempt in evaluating the pharmacokinetic behavior of gallic acid post topical application of the standardized ethanolic extract of *Chrysophyllum cainito* and *Mimusops elengi*.

MATERIALS AND METHODS

Chemicals and reagents

Nylon micro filter paper (0.45 µm) and gallic acid (98% purity, Figure 1) were obtained from Sigma-Aldrich Chemicals (St. Louis, USA). Clonidine hydrochloride (Arkamin Clonidine tablets, Unichem Laboratories Ltd., Mumbai, Figure 1) was purchased from the local market. Ethyl acetate, formic acid, orthophosphoric acid, HPLC grade solvents (ethanol, water and acetonitrile), potassium dihydrogen phosphate (KH_2PO_4) were obtained from Merck Specialities (Mumbai, India). All other chemicals used were of analytical grade. Ultra-pure water was obtained using a Milli-Q purification system (Millipore, USA). Glassware from Borosil was used throughout the study.

Experimental animals

In order to evaluate the pharmacokinetic profile of gallic acid from the ethanolic extract of *Chrysophyllum cainito* and *Mimusops elengi*, a study was conducted in Wistar rats. Healthy young adult male Wistar rats weighing between 220-240 g were procured from Haffkine Biopharmaceuticals, Mumbai, India. Rats were housed in polypropylene cages provided with sterilized rice husk bedding in a ventilated room of the animal house facility (CPCSEA/315; Ramnarain Ruia College, Mumbai, India) under standard experimental conditions with 20 ± 3°C ambient temperature, relative humidity (60 ± 5%) and 12 h dark/light cycle. All the animals were fed standard pellet diet (Amrut laboratory animal feed, India) and were provided water *ad libitum*.

Chromatographic conditions

A HPLC system consisting of two PU-1580 pumps (HG-1580-31), rheodyne injector (20.0 µL loop), photo diode array detector (MD-1510), Jasco-Borwin chromatography software version 1.50, Jasco, Easton, USA was used in this study. The separation was achieved on Cosmosil C_{18}-column (150×4.6 mm, 5 µm). The mobile phase [10 mM potassium

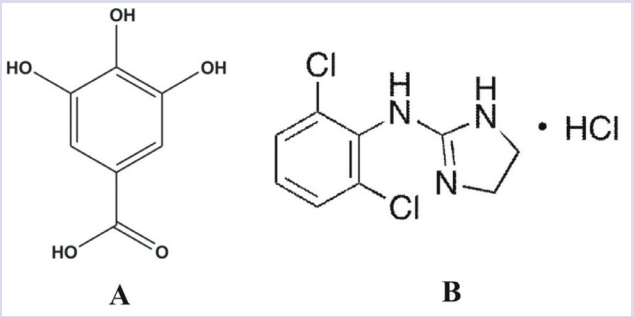

Figure 1: Structure of gallic acid (A) and clonidine hydrochloride (B)

dihydrogen phosphate (KH_2PO_4) in water: acetonitrile: orthophosphoric acid (95: 5: 0.05, v/v/v)] was delivered (isocratic mode) at a flow rate of 1.0 mL/min. After 30 min of equilibration, the samples (20.0 µL) were injected into the HPLC system. Peaks were recorded at 215 nm.

Standard solutions

Stock solutions of gallic acid (1000.0 µg/mL) and the internal standard (IS) clonidine hydrochloride (250.0 µg/mL) were prepared in methanol. From the stock solution, the working standard solutions of gallic acid with 0.5 µg/mL, 0.75 µg/mL, 1.0 µg/mL, 2.5 µg/mL, 12.5 µg/mL, 25.0 µg/mL and 50.0 µg/mL were prepared in mobile phase. IS at a concentration of 25.0 µg/mL was also prepared in mobile phase by diluting its stock solution. All the stock and working solutions were stored at 5 °C ± 3°C.

Sample preparation

Drug-free rat plasma was obtained from male Wistar rats in heparinised tubes. The plasma calibration standards of gallic acid (0.05 µg/mL, 0.075 µg/mL, 0.10 µg/mL, 0.25 µg/mL, 1.25 µg/mL, 2.50 µg/mL and 5.00 µg/mL) were prepared by adding 40.0 µL of working solution (composed of 20.0 µL gallic acid and 20.0 µL IS from respective working solutions) into 200.0 µL drug-free rat plasma. To this, ethyl acetate (2000.0 µL) was added to precipitate the proteins. The mixtures were shaken for 10 min on a rotary shaker. After the centrifugation at 4000 rpm for 10 min, the supernatant/ organic layer (2000.0 µL) was collected and transferred into evaporating tubes. The content was evaporated at 40°C under nitrogen stream in low volume evaporator. The residue was reconstituted with mobile phase (200.0 µL) and then 20.0 µL of the sample was injected into the HPLC system for analysis. Quality control (QC) samples were prepared separately in a similar way as calibration standards and the concentrations of 0.15 µg/mL, 0.75 µg/mL and 4.0 µg/mL of gallic acid in plasma samples were corresponding to low, medium and high QC, respectively.

Method validation

The developed HPLC method was validated in accordance with the bioanalytical guidelines for the parameters like selectivity, linearity, accuracy, precision, recovery and stability.[18]

Selectivity

Selectivity of the method was performed by comparing the chromatograms of the following samples. Gallic acid and IS were dissolved in mobile phase and injected into the HPLC system directly at LLOQ level, blank plasma, plasma sample spiked with gallic acid and IS at LLOQ level, and plasma sample of a rat at 1.00 h after the topical application of the ointment containing ethanolic extracts of *Chrysophyllum cainito* and *Mimusops elengi* spiked with IS. Parameters like Rt and absorption spectrum were assessed. The presence of any interference from exogenous or endogenous materials at the Rt of gallic acid and IS was observed.

Linearity

Every calibration standard concentration was assayed six times. After injecting all the processed calibration standard samples of various concentrations, the calibration curve was established in the range of 0.05-5.0 µg/mL. The peak areas of gallic acid (A_{GA}) and IS (A_{IS}) were recorded; the ratio of the values of area of gallic acid to the area of IS (A_{GA}/A_{IS}) and the concentrations of gallic acid were used to plot the calibration curve ($y = mx + c$).

Accuracy and precision

In order to assess the precision and accuracy of the method, three quality control samples of gallic acid at low (LQC: 0.15 µg/mL), medium (MQC: 0.75 µg/mL) and high (HQC: 4.0 µg/mL) concentrations were used and analyzed six times for each concentration on the same day (intra-day precision and accuracy) and on three consecutive days (inter-day precision and accuracy) using HPLC method. The precision and accuracy of the method was assessed by calculating the % CV and % nominal for each concentration level. The acceptance criteria for intra- and inter-day precision and accuracy were considered within ± 15% for all levels.

Recovery

The recoveries of gallic acid were determined by the analysis of three quality control samples; low (LQC: 0.15 µg/mL), medium (MQC: 0.75 µg/mL) and high (HQC: 4.0 µg/mL). The peak area ratio of gallic acid to IS (A_{GA}/ A_{IS}) spiked in blank rat plasma was obtained. For the reference material, the same concentration standard solution in mobile phase was injected directly to the HPLC system and the peak area ratio of gallic acid to IS (S_{GA}/ S_{IS}) in mobile phase was also obtained. The results of (A_{GA}/ A_{IS})/ (S_{GA}/ S_{IS}) were defined as the method recoveries and expressed in percent.

The extraction recoveries (%) of gallic acid were determined using the following method: The peak area of gallic acid spiked in blank rat plasma (A_{GA}) was compared to that of gallic acid directly dissolved in mobile phase and assayed by HPLC at the same concentration (S_{GA}); the extraction recoveries of internal standard were obtained by analyzing plasma samples spiked with IS at medium level of QC (0.75 µg/mL) using the same method. The experiments were repeated six times for each concentration.

Stability

The stability of gallic acid in plasma was assessed by analyzing six replicates of quality control samples at low (LQC: 0.15 µg/mL), medium (MQC: 0.75 µg/mL) and high (HQC: 4.0 µg/mL) concentrations during the sample storage and processing procedures. Freeze/thaw stability was determined after three freeze/thaw cycles (each cycle by storing the samples at -70 ± 5°C in a deep freezer for 24.0 h followed by thawing for 60 min at room temperature). The bench-top stability was assessed by exposing the spiked samples (collected after the extraction procedure) at room temperature for 4.0 h and 24.0 h. Long-term stability of the samples was evaluated for one month at -70 ± 5°C. The stability was determined by comparing the calculated concentration of the test samples with the nominal concentration of the freshly prepared sample. Analytes were considered stable at each concentration when the concentration differences between the freshly prepared samples and the test samples were found within ± 15%.

Pharmacokinetic study

Standardization of the extracts and preparation of the test samples

The ethanolic extracts of *Chrysophyllum cainito* and *Mimusops elengi* were standardized as previously reported.[11-12] The ointment of *Chrysophyllum cainito* and *Mimusops elengi* (each containing 20% ethanolic extract) was prepared as described previously.[11-12,19]

Experimental design

The protocol for pharmacokinetic study was approved by Institutional Animal Ethics Committee before commencement of the study (Approval No. DG-130624-04). Eighteen healthy male Wistar rats with the intact skin were selected for the pharmacokinetic study. Prior to the study, all the animals were acclimatized in the experimental room for a week. Approximately 24 h before the test, fur was removed (3 cm^2) by closely clipping the right and left area on the dorsal side of the trunk of each rat (1 patch on each side). Care was taken to avoid abrading the skin. Animals were randomly divided into two groups of nine animals each. Followed by the period of fasting (5 h), the ointment containing 20% ethanolic extract of *Chrysophyllum cainito* (equivalent to 0.71 mg gallic acid) and *Mimusops elengi* (equivalent to 0.61 mg gallic acid) was applied topically on the depilated region (0.25 g on each side) of the animals from respective groups after which food and water were given *ad libitum*.

A sparse sampling methodology was employed for the collection of blood samples from the rats as per our published reports.[20-22] Blood was withdrawn at 14 time intervals - 0.00 h, 0.15 h, 0.30 h, 0.45 h, 1.00 h, 1.50 h, 2.00 h, 2.50 h, 3.00 h, 4.00 h, 6.00 h, 8.00 h, 12.00 h and 24.00 h post application. Animals from both the groups were divided into three sets (6 animals per set). Rats from first set accounted for six samples and rats from second and third set accounted for five samples each including the pre-dose sample (0.00 h).

Blood samples of about 0.3 mL per sampling point were collected from the retro-orbital plexus of the rats using heparinized capillaries into heparinized micro-centrifuge tubes. After collection, blood samples were immediately centrifuged at 4000 rpm for 10 min. The supernatant plasma was transferred into sample vials and stored at -70±5°C until further analysis. The plasma samples were then extracted as described earlier. The difference was that 20.0 µL of the IS working solution was added to the above plasma before the addition of ethyl acetate (2000.0 µL) to precipitate protein and the residue was reconstituted in 200.0 µL of the mobile phase. All the plasma samples were assayed within two days after the application.

Statistical analysis

WinNonlin computer software (WinNonlin® 6.3, Phoenix™, USA) was used for the determination of the pharmacokinetic parameters C_{max} (maximum drug concentration), T_{max} (time to reach the maximum drug concentration), $t_{1/2}$ (half-life), K_{el} (terminal elimination rate constant), AUC_{0-24h} (area under the plasma concentration-time curve) and $AUC_{0-\infty}$ (area under the plasma concentration-time curve extrapolated to infinity to obtain last measurable plasma drug concentration).[21-24] All results are expressed as the arithmetic mean ± standard deviation. The statistical analysis, scientific graphing and data evaluation were performed using Excel-2007 (Microsoft, New York, USA).

RESULTS AND DISCUSSION

HPLC method development

Use of a suitable internal standard substance is the key for biological sample analysis. The internal standard should be stable, should not interfere with the analyte and should not be present in the sample matrix.[25-26] On this basis, in the present study, amongst various modern drugs analyzed, clonidine was selected as an internal standard (IS) during the HPLC analysis of plasma samples.

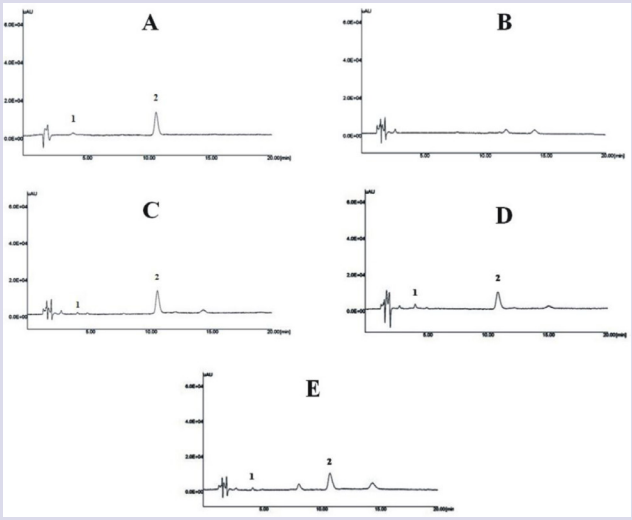

Figure 2: Representative HPLC chromatograms of gallic acid and IS. 1: gallic acid; 2: IS; A: Gallic acid (LLOQ level) and IS (2.5 µg/mL) in mobile phase; B: Blank plasma; C: Plasma sample spiked with gallic acid (LLOQ level) and IS (2.5 µg/mL); D: Plasma sample of a rat at 1.00 h after the topical application of the ethanolic extract of *Chrysophyllum cainito* spiked with IS (2.5 µg/mL); and E: Plasma sample of a rat at 1.00 h after the topical application of the ethanolic extract of *Mimusops elengi* spiked with IS (2.5 µg/mL).

The main aim of development of HPLC method was to achieve proper separation of gallic acid and IS with the reasonable Rt and sharp peak shape. In a previously published report, the mobile phase used to determine gallic acid in plasma by HPLC was comparatively complex and gave high Rt,[27] whereas, a solvent system consisting of 10 mM potassium dihydrogen phosphate in water: acetonitrile: orthophosphoric acid (95: 5: 0.05 v/v/v) provided satisfactory results in terms of reasonable run time, suitable Rt and separation of gallic acid and IS with sharp peak. Moreover, there was no interference of other endogenous compounds from plasma or plant matrix at the Rt of gallic acid and IS. Under the experimental conditions described above, the lower limit of quantification (LLOQ) of gallic acid in plasma was found to be 0.05 µg/mL.

Method validation

Selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. The presence of any potential interfering endogenous substances in a biological matrix was assessed at LLOQ level (0.05 µg/mL) of gallic acid. Selectivity of the method was performed by comparing the chromatograms of the following samples: gallic acid (LLOQ level) and IS (2.5 µg/mL) dissolved in mobile phase and injected to HPLC system directly, blank plasma, plasma sample spiked with gallic acid (LLOQ level) and IS (2.5 µg/mL), and plasma sample of a rat at 1.00 h after the topical application of the ointment containing ethanolic extract of *Chrysophyllum cainito* and *Mimusops elengi* spiked with IS (2.5 µg/mL). The representative HPLC chromatograms at 215 nm are shown in Figure 2A, 2B, 2C, 2D and 2E, respectively. It was observed that gallic acid and IS peaks were well shaped in the mobile phase, in spiked plasma samples as well as those detected from the plasma sample of rats treated with the ethanolic

extracts of *Chrysophyllum cainito* and *Mimusops elengi* at 1.00 h post-application. No interfering peaks were observed at the Rt of gallic acid and IS. The average Rt of gallic acid and IS was 4.21 min and 10.74 min, respectively. These observations indicated that the method had adequate selectivity.

Calibration curve

The HPLC method was validated for gallic acid in rat plasma over the concentration range of 0.05-5.0 µg/mL. A calibration curve prepared over this concentration range was found linear with the average correlation coefficient 0.999 in plasma. The typical regression equation was found to be y = 0.84539 (± 0.0516) x + 0.0018 (± 0.0001), (r^2 = 0.999), y representing the peak area ratio of gallic acid to IS and x representing the concentration of gallic acid in plasma.

Precision and accuracy

The intra- and inter-day precision and accuracy of the method were evaluated using the three quality control samples (LQC: 0.15 µg/mL), medium (MQC: 0.75 µg/mL) and high (HQC: 4.0 µg/mL) and the results are summarized in Table 1. The mean value of the accuracy was found within ± 15% of the theoretical value and the precision around the mean value was found less than 15% of the relative standard deviation (% CV). The data of the accuracy and precision in the assay were within the acceptance criteria.[18] Therefore, the results indicated that this method had good accuracy and precision.

Recovery

The method recovery was evaluated by comparing the peak area ratio of gallic acid and IS obtained from the extracted samples at the three quality control levels with the standard solutions of equivalent concentrations. The method recovery was found to be 84.24-88.05%. Simultaneously, the extraction recoveries of gallic acid and IS were determined by comparison of the peak area of the extracted samples at three quality control levels with the standard solutions of equivalent concentrations individually. The mean extraction recoveries for gallic acid and IS were between 78.46 and 86.92%. There was also no significant difference in the extraction recoveries of gallic acid at the three quality control levels indicating that the method for plasma handling was effective for extracting the gallic acid.

Stability

The stability experiments were carried out by analyzing three quality control samples to test the samples under all possible conditions that they might experience after collecting and prior to analysis. It was found that gallic acid was stable in plasma for three cycles when stored at -70°C and thawed to room temperature, which was evident by the facts that the calculated concentration after treatment was very near to the normal concentration. Bench top stability was evaluated at room temperature because the post-treatment of the samples was carried out under this condition. Gallic acid in rat plasma was stable at ambient temperature for 4.0 h and 24.0 h as well as after storage at -70°C for one month. Moreover, it was found that the loss of IS was also within the acceptable range. These results suggested that gallic acid and IS were stable under the experimental conditions of the analytical runs (Table 2).

Table 1: Validation data on intra- and inter-day precision and accuracy using quality control samples of gallic acid from rat plasma

Concentration of gallic acid spiked in plasma (µg/mL)	Mean calculated concentration of gallic acid (µg/mL)	Precision (% CV)	Accuracy (% Nominal)
	Intra-day		
LQC (0.15)	0.16 ± 0.001	**0.687**	**105.38**
MQC (0.75)	0.73 ± 0.010	**1.366**	**97.02**
HQC (4.00)	4.28 ± 0.205	**4.790**	**106.96**
	Inter-day		
LQC (0.15)	0.16 ± 0.001	**0.864**	**105.72**
MQC (0.75)	0.74 ± 0.023	**3.116**	**98.24**
HQC (4.00)	4.24 ± 0.157	**3.700**	**105.87**

Data expressed as mean ± SD, n = 6.

Table 2: Stability of the quality control samples of gallic acid from rat plasma spiked with IS.

Concentration of gallic acid spiked in plasma (µg/mL)	Mean calculated concentration of gallic acid (µg/mL)	Precision (% CV)	Accuracy (% Nominal)
	Freshly prepared samples		
LQC (0.15)	0.16 ± 0.004	2.681	106.80
MQC (0.75)	0.71 ± 0.040	5.556	95.07
HQC (4.00)	3.89 ± 0.186	4.787	97.21
	Freeze and thaw (three cycles)		
LQC (0.15)	0.14 ± 0.003	2.280	96.03
MQC (0.75)	0.78 ± 0.041	5.253	103.87
HQC (4.00)	3.89 ± 0.080	2.071	97.12
	Benchtop (for 4.0 h at room temperature)		
LQC (0.15)	0.16 ± 0.004	2.432	103.75
MQC (0.75)	0.74 ± 0.030	4.070	98.54
HQC (4.00)	3.92 ± 0.118	3.011	98.02
	Benchtop (for 24.0 h at room temperature)		
LQC (0.15)	0.16 ± 0.001	0.864	105.72
MQC (0.75)	0.74 ± 0.023	3.116	98.24
HQC (4.00)	4.22 ± 0.072	1.702	105.52
	Long term (at -70°C for one month)		
LQC (0.15)	0.16 ± 0.001	0.896	107.08
MQC (0.75)	0.79 ± 0.023	2.941	105.52
HQC (4.00)	3.92 ± 0.042	1.060	97.99

Data expressed as mean ± SD, n = 6.

Application of the developed HPLC method to pharmacokinetic study of gallic acid from the plant extracts

The method was successfully applied to the determination of gallic acid in plasma obtained from the rats post topical application of the ointments containing 20% ethanolic extract of *Chrysophyllum cainito* and *Mimusops elengi*, respectively. The pharmacokinetic study was performed by determining drug levels in plasma up to 24.0 h post-application of both the plant extracts. The blood was withdrawn from the respective set of animals as per their allotted time points. The blood samples were processed and were further analyzed using validated HPLC method. For both the plant extracts, it was observed that the concentration of gallic acid gradually increased from 0.15 h and reached its maximum at 1.00 h post-application. At 8.0 h, the concentration of gallic acid was found to be least, showing elimination at 12.0 h post-application. Plots of the mean plasma drug concentration-time profile curve for gallic acid from the ethanolic extracts of *Chrysophyllum cainito* and *Mimusops elengi* are depicted in Figure 3A and Figure 3B, respectively.

Pharmacokinetic analysis was performed by a non-compartmental approach. The area under the concentration versus time curve (AUC_{0-t}) was calculated by Linear-Log Trapezoidal rule from zero to the last measured concentration. Data on some major pharmacokinetic parameters calculated for gallic acid from both the plant extracts using WinNonlin software programme have been summarized in Table 3.

The pharmacokinetic parameters for gallic acid resulted after the topical application of the ointments containing 20% ethanolic extracts of *Chrysophyllum cainito* and *Mimusops elengi* and some of the reports on pharmacokinetics of gallic acid from herbal extracts following different route of administration[13-14] suggest that the absorption-elimination of plant-based markers differs from plant-to-plant on the basis of their route of administration. Thus, in this the pharmacokinetic profile of gallic acid after the topical application of the ethanolic extracts of *Chrysophyllum cainito* and *Mimusops elengi* has been determined in rats. The detection of gallic acid in rat plasma from the ethanolic extracts of *Chrysophyllum cainito* and *Mimusops elengi* as well as available reports on its therapeutic potential prove that when the ointment containing these two plant extracts are topically applied to rats, absorption of phytochemical constituents like gallic acid takes place which probably facilitates the biochemical changes leading to the wound healing activity. This study thus supports the wound healing potential of the ethanolic extract of *Chrysophyllum cainito* and *Mimusops elengi*.

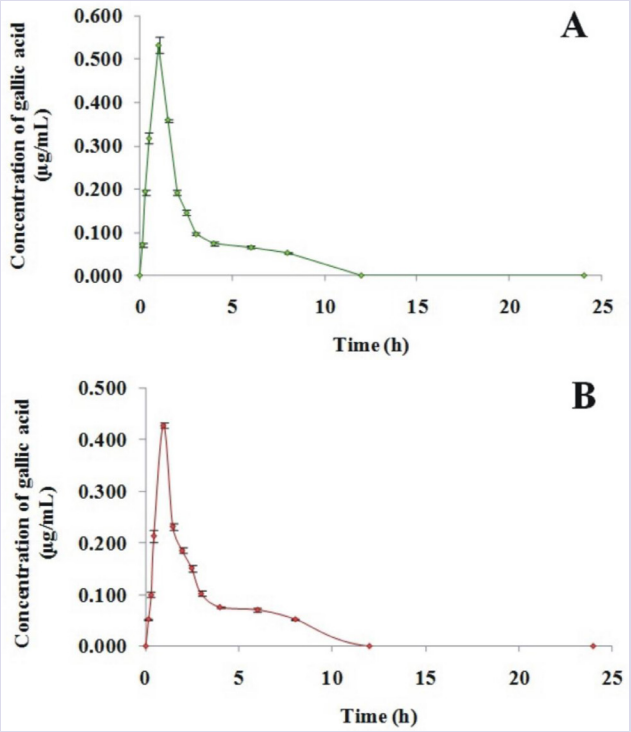

Figure 3: Mean plasma concentration-time curve showing the absorption elimination of gallic acid. A: Mean plasma concentration-time curve showing the absorption elimination of gallic acid after the topical application of the ethanolic extract of *Chrysophyllum cainito* and B: Mean plasma concentration-time curve showing the absorption elimination of gallic acid after the topical application of the ethanolic extract of *Mimusops elengi*.

Table 3: Pharmacokinetic parameters for gallic acid after the topical application of the ointments containing ethanolic extract of *Chrysophyllum cainito* and *Mimusops elengi* in rats.

Pharmacokinetic Parameters	*Chrysophyllum cainito*	*Mimusops elengi*
C_{max} (µg/mL)	0.53 ± 0.019	0.43 ± 0.006
T_{max} (h)	1.00 ± 0.000	1.00 ± 0.000
K_{el} (h^{-1})	0.10 ± 0.004	0.12 ± 0.004
$t_{1/2}$ (h)	7.32 ± 0.311	5.85 ± 0.254
AUC_{0-24} (µg/mL*h)	1.09 ± 0.042	0.92 ± 0.035
$AUC_{0-\infty}$ (µg/mL*h)	1.63 ± 0.073	1.36 ± 0.052

C_{max}: Maximum drug concentration, T_{max}: Time to reach the maximum drug concentration, K_{el}: Terminal elimination rate constant, $t_{1/2}$: Half-life, AUC_{0-24h}: Area under the plasma concentration-time curve, $AUC_{0-\infty}$: Area under the plasma concentration-time curve extrapolated to infinity to obtain last measurable plasma drug concentration. Data expressed as mean ± SD, n = 6.

CONCLUSION

The present study proves the bioavailability of gallic acid and establishes the pharmacokinetic parameters for gallic acid after the topical application of the ethanolic extract of *Chrysophyllum cainito* and *Mimusops elengi* in rats. The study also demonstrates the feasibility of using HPLC as a tool for evaluation of the pharmacokinetics of bioactive markers from the plant extracts. The detection of gallic acid in rat plasma from the ethanolic extracts of *Chrysophyllum cainito* and *Mimusops elengi* suggests that gallic acid might be responsible as one of the phytochemical constituents showing wound healing activity of the plant extracts, which is also supported by other published reports on its therapeutic potential. The findings of this study might help to provide useful evidence for the clinical applications of these plant extracts.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ABBREVIATIONS

CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; **CV:** Coefficient of variation; **HPLC:** High Performance Liquid Chromatography; **HQC:** High quality control; **IS:** Internal standard; **LLOQ:** Lower limit of quantitation; **LQC:** Low quality control; **MQC:** Medium quality control; **Rpm:** Revolutions per minute; **Rt:** Retention time; **SD:** Standard deviation; **USFDA:** United States Food and Drug Administration.

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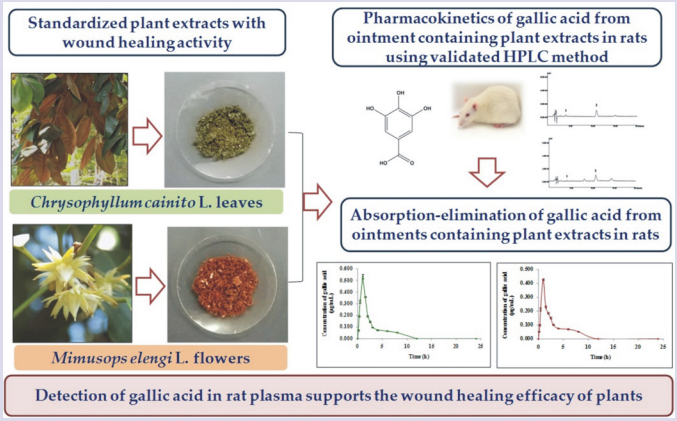
PICTORIAL ABSTRACT



SUMMARY

- The wound healing activity of the ointments containing standardized ethanolic extract of *Chrysophyllum cainito* leaves and *Mimusops elengi* flowers has been evaluated scientifically through our previous studies using *in vivo* excision wound rat model.
- In the present pharmacokinetic study, the absorption - elimination of gallic acid from the ointments containing 20% standardized ethanolic extract of both the plants was studied in rats using validated HPLC method.
- The detection of gallic acid in rat plasma post topical application of the ethanolic extracts of both the plants proves its absorption which probably facilitates the biochemical changes and thus exhibits wound healing activity of the plant extracts.

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Dr. Deepti Gurjar has pursued her Ph.D. in Bioanalytical Sciences at Ramnarain Ruia Autonomous College, Mumbai, Maharashtra, India. She has worked as a Senior Research Fellow on the project funded by Department of Biotechnology, Government of India in the area of standardization of some medicinal plants and evaluation of their therapeutic efficacies. She has nine publications and presented her work in this field at National and International Conferences.

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