Pharmacokinetic Studies on Rabbits as a Tool to Evaluate the Quality of *Ricinus Communis*

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Abstract

Pharmacokinetics is a study of movement of a drug in our body. Usually, a modern drug will consists of the ingredient and excipients. It is relatively easy to trace the pharmacokinetics of the modern drug. In case of herbal medicines, usually most of the products over the counter are polyherbal. There is no component clearly said to be the active principle responsible for the dedicated medicinal effect. Hence, one needs to establish some phytochemical markers for the identification of the herb(s) in the blood, which can be used to trace the fate of the drug in the living system. This will help us to establish the absorption elimination process of the herb. The markers thus obtained, need not be the active principle or constituent of the drug. This will help to decide the dosage regime and the therapeutic window of the drug. The present work was undertaken with an objective to establish a phytochemical marker for the detection of Ricinus communis in the blood of rabbits and to establish the pharmacokinetics of the herb. I was able to identify one component from Ricinus communis in the plasma of the rabbits fed with the leaf powder of Ricinus communis as a dose. The phytochemical could be detected using HPLC at Rt 5.707 minutes for Ricinus communis. This phytochemical was used to establish the pharmacokinetics of leaf powder of Ricinus communis.

***Keywords**: markers, Ricinus communis, RP-HPLC, Pharmacokinetics.*

Introduction

Pharmacokinetics is the science of quantitative actions between a biological organism and pharmacology within it. The word pharmacokinetic was first introduced by Dust in his book "Der Blustpiegel" (Blood vessel) (M.Gaibaldi, 1991). In 1933, Gehlen expressed his idea that intravenously administered drugs follow the function of time. He found out that Tmax is independent of the dose (H.M. Abdou, 1989). In 1937, Teorell made one of the most important contributions to the field of pharmacokinetics with his famous manuscript "Kinetic of distribution"of substances administered to the body (H.M.Abdou 1993). In 1949, Druckery and Kupfmuller's monograph "Dosis and Wikung"(Dose and its effect) stated a complete theory of pharmacokinetics and its modern aspect like effect kinetics, system kinetics using various electrical analog circuits to visualize time courses (H.M.Abdou 1989). New drug cannot be a subject of licencing application nor be put in the market, unless pharmacokinetic data are available. Pharmacokinetics is one of the most important and essential foundations of clinical pharmacokinetics. Various methods for the estimation of a specific component in biological fluid like Liquid chromatography, Gas chromatography, High pressure Liquid Chromatography are used.

Herbal medicine finds vast application in clinical studies. It is necessary to device a series of experiments which are capable of determining the bioavailability of herbal medicines in biological fluids. Drug concentrations are determined in the systemic circulation in order to describe their kinetics within the body. The major biological fluid (matrices) analysed are blood, plasma, serum, tears, cerebrospinal fluid (CSF), saliva and urine. Blood, plasma, serum and urine are most commonly investigated. Saliva has been used as a potential substitute for plasma because it can be obtained by non-invasive techniques and concentration of the drug in saliva can be correlated to that in plasma. Objective of analytical method development is to develop such a method that will detect one or more relative marker compounds, which will be helpful in *invivo* study.

Materials and Methods

Ricinus communis (Linn), of family Euphorbiaceae, have been used in traditional medicine for abdominal pain, arthritis, backache, sciatica, constipation, gall bladder pain etc. (S.Khan et al, 2017). Leaves of *Ricinus communis were* collected from Thane district of Maharashtra. The leaves were thoroughly washed with water to remove dust and other extraneous matter, the excess of water was absorbed by spreading the plant material over filter paper for three days in shade away from sunlight. The filter paper was replaced daily. Herbaria were prepared and were sent for authentication to NBRI Lucknow. Leaves that turned yellow were discarded. The rest of the leaves were then placed in a pre-set oven at 45±5°C. The plant material was allowed to dry for four days. Immediately after drying, it was powdered using an electric mixer grinder and sieved through a BSS mesh NO 85 sieve. The sieved powdered was stored in commercially available airtight polythene container with date and time of collection. This powdered plant material was used for further work.

Animal Model: Animals used for the investigation were New Zealand albino rabbits, procured from Haffkin Biopharmaceutical Ltd Mumbai, ranging between 2± 0.2 kg. The animals were subjected to acclimatization, for which they were kept in a separate quarantine room for seven days. The animals were provided with drinking water *ad libitum* and were fed on commercially available feed supplied by Amrit feed.

Invivo Study

Pilotstudy - One healthy New Zealand strain Albino rabbit weighing between2.0±0.2 kg was used in this study. Oral route administration was selected for the present study the rabbit was starved for 18 hrs prior to the administration of the plant material. Blank sample (zero) was collected from the rabbit and was fed orally with 1g/kg body weight of *Ricinus communis* leaf powder in 20 cm^3 of distilled water by using gavage no 10. The blood samples were collected at an interval of 30, 60, 120, 240, 480 and 720 minutes post dose administration. 3cm^3 of blood was collected in sterile heparinised appendorff tubes. Chromatographic conditions remained the same as explained in the *invivo* study.

Main Study

Pharmacokinetic study was carried out using HPLC method. HPLC system used in the present study was JASCO PU-1580 with JASCO MD -1410 PDA detector. The column used in the present study was COSMOSIL 5C-18-MS, SIZE 4.6X150 mm. Manufacture No K01016.

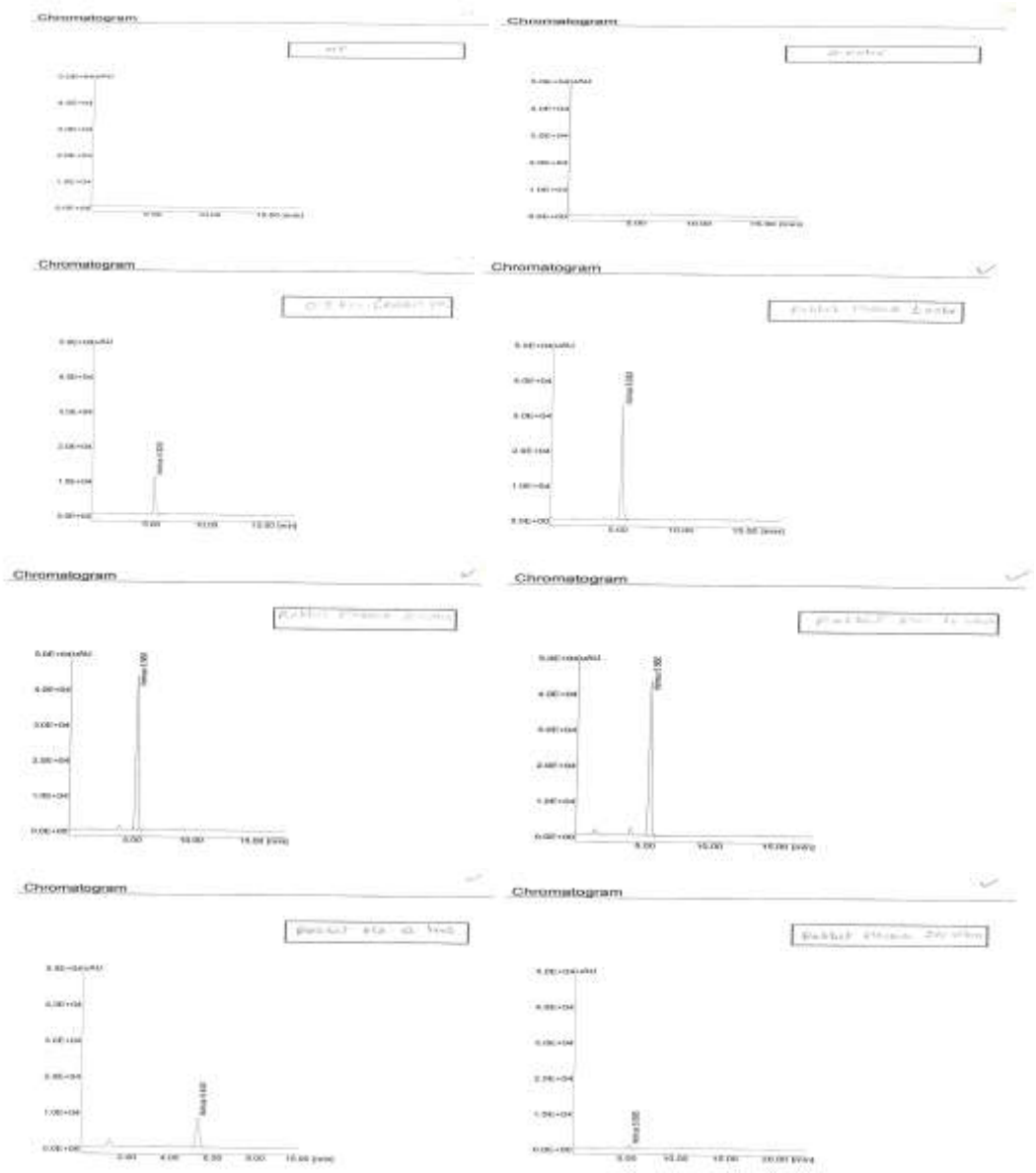
Various mobile phases were tried like Methanol, Acetonitrile, and Distilled water. However, best separation of *Ricinus communis* leaf marker was found in ACN: D/W (0.5:95) + (100 mg of Hexone sulfonic acid/ 100 ml of mobile phase). The Rt of marker was 5.707.

Blood was removed from the rabbit's ear, before administering the oral dose. Blood was centrifuged at 4500 rpm for 15 minutes to separate plasma from the blood. The plasma was used for finding the linear response of marker. Concentrations ranging from 5, 20, 30, 100, 200, 500, 1000 µg/ml were made. The percent nominal ranged from 90.35 to 112.94, with RSQ at 0.999.

Oral dose of 1g/kg body weight was given to the same rabbit. 3cm^3 of blood was removed in sterile, heparinised Eppendorf tube at the intervals of 0.50, 1.00, 2.0, 4.0, 12.0, 24.0 hours of post dose. The Eppendorf tubes were centrifuged at 4500 rpm for 15 minutes, and 0.5cm^3 of plasma was separated in 10.00cm^3 clean dry stoppered test tubes. 10.00 cm^3 of dichloromethane (DCM) was added to every test tube and the test tubes were shaken for 10 minutes. The test tubes were centrifuged for 10 minutes at 400rpm. Supernatant aqueous layer was removed carefully using hypodermic syringe. 8.00 cm^3 of Dichloromethane was transferred to a low volume evaporating tubes. Tubes were transferred to a water bath, pre-set at 50°C for the evaporation of the organic layer. Rapid evaporation was done under nitrogen flow. After evaporation, the residue was reconstituted in 500µL of mobile phase. 20µl of reconstituted extract was injected in HPLC system.

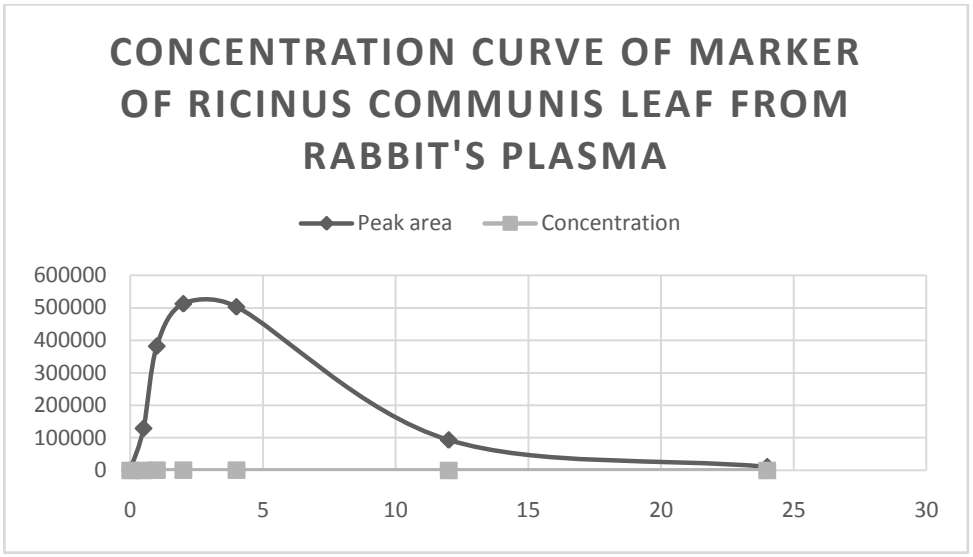
Observations

Ricinus communis leaf marker was not observed at 0.00 hours.However, its concentration in plasma increased gradually and reached its maximum at 2.00 hours post dose. It then decreased gradually thereafter. At 24hours, the concentration was found out to be the least.

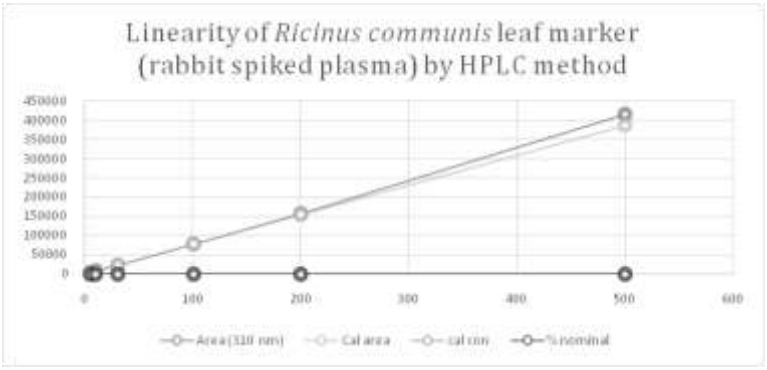


Readings of Marker in Tabular Form

Sampling hours	Peak area	Concentration
0	0	0
0.5	129131	178.21
1	381995	533
2	512451	718.04
4	502938	702.7
12	93293	127.93
24	10794	12.17



Linearity of *Ricinus communis* leaf marker (rabbit spiked plasma) by HPLC method				
CON	**Area (310 nm)**	**Cal area**	**cal con**	**% nominal**
5	4204	3873.134682	4.61	92.21
10	8511	7746.269364	10.17	101.71
30	23910	23238.80809	30.05	100.17
100	77639	77462.39364	99.41	99.41
200	157937	154925.3873	203.07	101.54
500	416000	387313.4682	536.22	107.24



Conclusion

The pharmacokinetic study of *Ricinus communis* leaf showed a typical absorption elimination patter. This study demonstrated the probability of developing the methods to detect markers of other plants in biological matrix using similar approach.

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