RESEARCH ARTICLE

In Vitro Drug Release and Study of Anti-inflammatory Effect of Rasna Saptak Kwath

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Abstract

Aim: Rasna Saptak Kwatha is an Ayurvedic formulation used orally to relieve inflammation and pain in arthritis. It has seven ingredients, namely, *Pluchea lanceolata, Tribulus terrestris, Tinospora cordifolia, Boerhavia diffusa, Ricinus communis, Cedrus deodara,* and *Cassia fistula. The Zingiber officinale* is used as additives at the time of administration. **Materials and Methods**: All these herbs were mixed together and making decoction and hydroalcoholic extraction of *Rasna Saptak Kwatha churna*. After that permeability of drug was checked as a membrane. **Result and Discussion**: The *in vitro* experiment results showed that the drug is permeating through the skin. In the carrageenan edema model, hydroalcoholic extract showed better inhibition in comparison to aqueous extract. **Conclusion**: It can be concluded from the study that extracts could be served or can be modified for topical or transdermal drug delivery system.

Key words: Anti-inflammatory, Arthritis, *Rasna Saptak Kwath*

INTODUCTION

Arthritis is characterized by inflammation of the flexible joints, leading to joint swelling, stiffness, etc., which can eventually result in cartilage damage. *Rasna Saptak Kwath*[1] an ayurvedic polyherbal formulation is prescribed to the patient in the form of decoction for treatment of arthritis. *Rasna Saptak Kwatha* (RSK) is a formulation of seven herbs, namely, *Pluchea lanceolata, Tribulus terrestris, Tinospora cordifolia, Boerhavia diffusa, Ricinus communis, Cedrus deodara,* and *Cassia fistula,* while the eighth drug, i.e., *Zingiber officinale* is used as additives at the time of administration. To treat symptomic conditions such as inflammation and pain, arthritic patients need oral as well as local treatment. RSK orally gives satisfactory result to arthritis patients, but at the same time, patient needs local treatment also. The bitter taste of RSK, tedious process of making decoction, and short life of decoction all these reasons together compel the patients to give up this treatment patients' need ready to use treatment for their disorders. Hence, there is a need of time to provide this formulation in topical or transdermal dosage form. To fulfil this aim, first, there is a need to perform a evaluation study which will make it confirm whether the drug is permeating through the skin or whether it will give any anti-inflammatory effect or not. Therefore, two evaluation studies were performed. First, *in vitro* drug release study and second anti-inflammatory study. In this preliminary study, the aqueous and hydroalcoholic extracts of *Rasna saptak kwath churna* evaluated for its skin permeability through *in vitro* drug release study and anti-inflammatory study through carrageenan edema model.

MATERIALS AND METHODS

All the ingredients of formulations were procured from local Ayurvedic market (Goladinanath, Varanasi, Uttar Pradesh, India) except *Eranda and Guduchi*, which were collected from

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botanical garden of Banaras Hindu University. Plant materials were authenticated in the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University (BHU), Varanasi, India, and voucher specimens were kept in the Museum of the Laboratory of Dravyaguna, BHU, Varanasi, India. These specimens were provided with a specific code or number and these numbers are, for Rasna DG/17-18/144, Guduchi DG/17- 18/145, Gokshura DG/17-18/146, Punarnava DG/17-18/147, Eranda DG/17-18/148, Devdaru DG/17-18/149, Aragvadha DG/17-18/150, Sunthi DG/17-18/151.respectively.

Preparation of plant extract

The whole plant material of RSK [Table 1] was dried and grind to prepare coarse powder (mesh size # 10), and then, it was collectively, i.e., RSK churna extracted through two different processes. One of the extractions was done in hydroalcoholic (50:50), i.e., distilled water and ethanol media through Soxhlet process at temperature 80°C and extract was concentrated through Rota evaporator. Another extraction was done through decoction method where the temperature was maintained at 70–80°C. These two extracts were further concentrated and dried through evaporation method where the temperature was maintained at 40–45°C. The dried extracts were obtained and packed.

Estimation of gallic acid in extracts

Selection of common solvent

After assessing the solubility of gallic acid in different solvents, it was found that gallic acid dissolved completely in methanol. Therefore, methanol has been selected as a common solvent for developing spectral characteristics.

Selection of wavelength

A representative spectra of gallic acid in methanol were obtained through ultraviolet (UV)-spectroscopy. The dilution of gallic acid in methanol was obtained to the concentration of 10 µg/ml. The gallic acid solution was scanned in UV range (200–400 nm) in 10 mm cell against solvent blank. The study of spectrum revealed that gallic acid shows absorbance at 261 nm.

Preparation of standard stock solution and study of Beer–Lambert's Law

The standard stock solutions of gallic acid were prepared by dissolving 50 mg of drug in methanol, and final volume was adjusted with the same solvent in 50 mL of volumetric flask to get a solution containing 1000 µg/mL. The further dilution of standard stock solution was done with methanol to prepare the aliquots of working stock solutions to get a concentration in the range of 2–10 µg/ml for gallic acid in methanol. The absorbance of resulting solution was measured at their respective λ_{max}. A calibration curve as concentration versus absorbance was constructed to study the Beer–Lambert's Law and regression equation [Figure 1].

Chemical

Ethanol, methanol, and phosphate buffer saline 7.4 pH were of analytical grade purchased from Science house, Varanasi, India. Gallic acid procured from Natural remedies, Bengaluru, India (purity >97%).

Experimental animals

The Wister rats weighing between 150 to 200 g were procured from the Central Animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi, and maintained under constant conditions (temperature 25 ± 2°C, humidity 40–60%, 12 h light/12 h dark cycle). During maintenance, the animals received a diet of food pellet supplied from the animal house and water *ad libitum*. This experiment was approved by the Institutional Animal Ethics Committee, Institute of Medical Sciences, Banaras Hindu University, Varanasi (IAEC No. 542/02/ab/CPCSEA 27.12.2012).

Table 1: Ingredients present in *Rasna Saptak Kwath*[1]

Plant name	Scientific name	Part used	Amount taken (g)
Rasna	*P. lanceolata* Clarke.	Leaf	100
Gokshura	*T. terrestris* Linn.	Fruit	100
Guduchi	*T. cordifolia* (Wild) Miers	Stem	100
Punarnava	*B. diffusa* Linn.	Root	100
Eranda	*R. communis* Linn.	Root	100
Devdaru	*C. deodara* (Roxb.) Loud	Stem	100
Aragvadha	*Cassia fistula* Linn.	Fruit	100
Sunthi	*Zingiber officinalae* Rosc.	Rhizome (dry)	100

P. lanceolata: Pluchea lanceolata, T. terrestris: Tribulus terrestris, T. cordifolia: Tinospora cordifolia, B. diffusa: Boerhavia diffusa, R. communis: Ricinus communis, C. deodara: Cedrus deodara, C. fistula: Cassia fistula, Z. officinalae: Zingiber officinalae

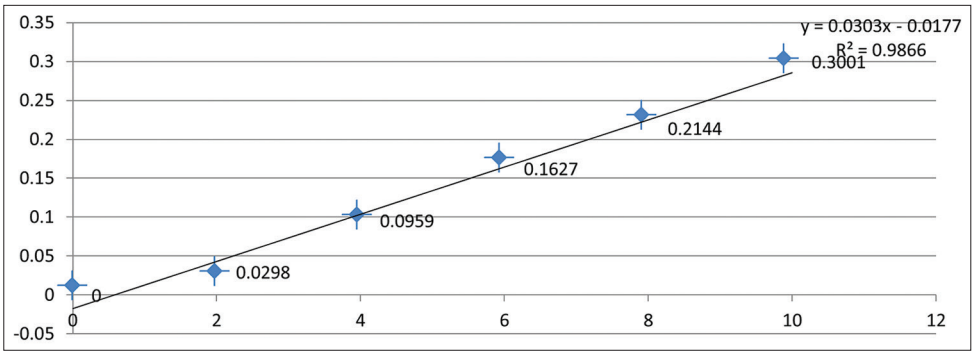

Figure 1: Calibration curve of gallic acid

In vitro drug release of extract

Preparation of rat skin[2]

Hairs from the abdominal side of male albino rat weighing (150–200 g) were removed by a commercially available hair remover (Veet®). The animals were then sacrificed. The full thickness skin of the abdomen was surgically removed and the adhering subcutaneous fat was carefully cleaned. Any trace of fat adhering was finally removed by wiping with cotton soaked in isopropyl alcohol. Finally, skin was rinsed with distilled water then with saline [Figure 2]. The skin was stored at −20 °C soaked in saline and tightly covered with aluminum foil till further use within not more than a week from storage to avoid skin damage or any variation in pore size.

Transdermal permeation studies using Franz-diffusion cell

In vitro diffusion study was conducted through Franz-diffusion cell[3] [Figure 2]. Excised skin was then placed between the donor and receptor compartments of the cells. The skin was placed with the stratum corneum facing the donor compartment. Approximately 25ml of phosphate buffer (PBS, pH 7.4) was placed in the receptor compartment. The temperature was maintained at 32 ± 0.5°C using a thermostatic water bath, and the rotation speed was set at 600 rpm during the experiment. The donor compartment contained 100 mg of extract. 5 ml of the receptor medium were withdrawn and replaced with an equal volume of freshly prepared medium at predetermined time intervals. The study was conducted for 24 h of time. The amounts of gallic acid permeated through the skin into the receptor medium were determined using a UV spectrophotometer at 261 nm.

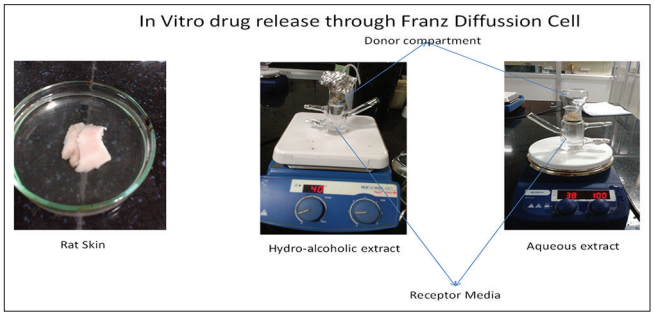

Figure 2: Franz diffusion study for extract

Dose fixation and schedule

Decoction and extract were given in the dose 90 mg and 108 mg, respectively, and this dose was decided per the table of Paget and Barnes.[4]

Carrageenan-induced paw edema model

Paw edema was induced[5] by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into subplantar tissues of the left hind paw of each rat. Rats were divided into four groups; each group consists of six animals.

- Group control: Carrageenan control.
- Group standard: Diclofenac sodium 1.0%(10 mg/kg) as reference standard.
- Group A: Hydroalcoholic extract (540 mg/kg).
- Group B: Aqueous Extract (450 mg/kg).

The paw thickness was measured before injecting the carrageenan and after 2 h., 4 h., 6 h, and 8 h. The test animals weighing 150–200 grams were fasted for 24 h before the experiment with free access to water only. Approximately 0.1 mL of 1% carrageenan solution was injected into the plantar side of the right hind paw of the rats. Paw volume was measured and noted immediately after the carrageenan injection. Afterward, respective dose of both the extract was applied to the plantar surface of the right hind paw. The extracts were gently rubbed 50 times with the use of index finger. As for the standard, test animals of the control group did not received any medicament and 1.0% diclofenac sodium gel applied to the standard group in the same way as the other groups. Paw volume was measured at 2 h interval for a maximum of 8 h after the administration of carrageenan solution using a plethysmograph.

Statistical analysis

All values are expressed as mean ± standard error of the mean. *P* value calculated by comparing with control by

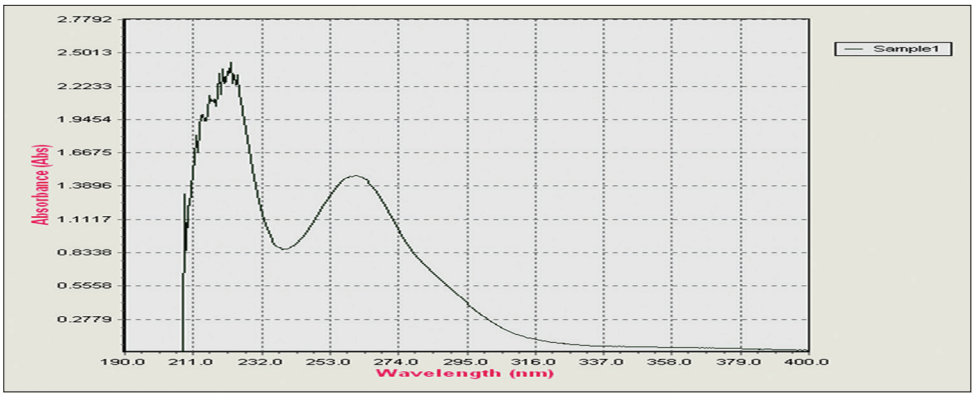

Figure 3: Standard gallic acid

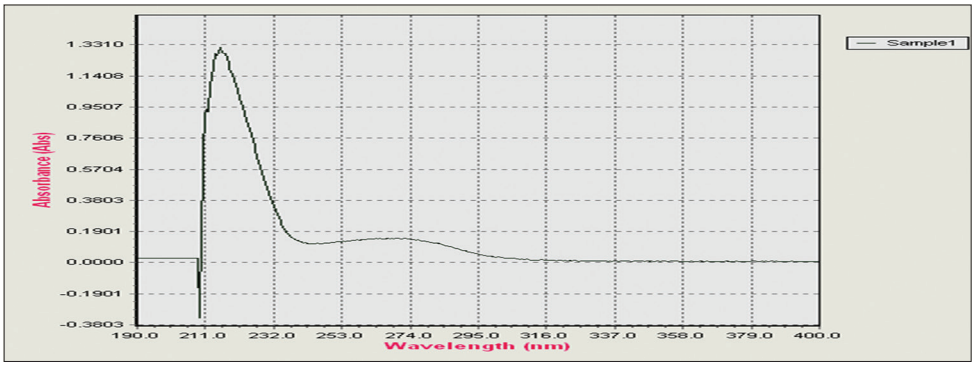

Figure 4: Gallic acid in receptor media (hydro-alcoholic extract)

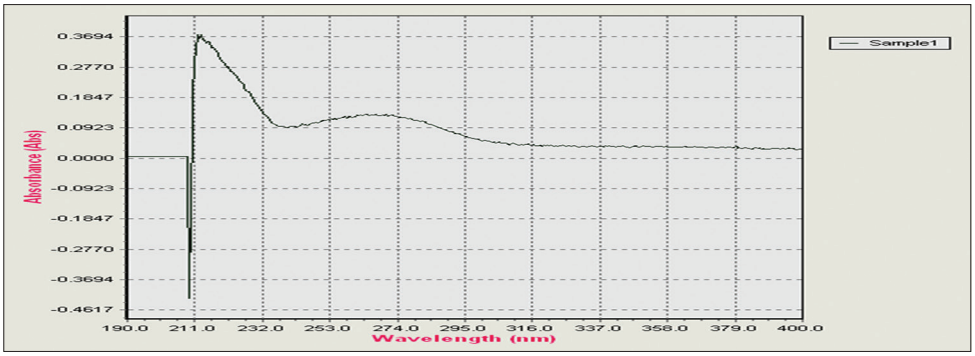

Figure 5: Gallic acid in receptor media (aqueous extracts)

ANOVA followed by Bonferroni post-test. *P* values lower than 0.05 were considered statistically significant.

RESULTS AND DISCUSSIONS

Presence of gallic acid in extracts

5 mg extract was transferred to 50 ml volumetric flask and dissolved in methanol, and final volume was made up with methanol. The sample solution was then filtered through Whatman filter paper No.41. From the above solution, 100 ml of the solution was taken and diluted to 10 ml with methanol to get final concentration and absorbance was taken at 261 nm for the presence of Gallic acid. Finally, with the calibration curve, concentration of gallic acid was determined in extracts. Figures 3-5 show the standard gallic acid spectrum, gallic acid in hydroalcoholic, and aqueous media, respectively.

The present investigations reveal that both extracts have gallic acid 8% and 4% in hydroalcoholic and aqueous extract, respectively. Gallic acid was taken as a standard to know the concentration passing through the skin. The concentration of gallic acid was found in receptor media but in very less amount which is shown in Figures 6 and 7 for hydroalcoholic and aqueous extract, respectively, i.e., 33% and 29%. For anti-inflammatory study, Table 2 depict data related to the effect of test drugs

Table 2: Effect of test drugs on carrageenan-induced paw edema in rats

Groups	Percentage change in paw volume at different time interval after Carrageen injection								
	0 h	2 h	change	4 h	change	6 h	change	8 h	change
C	18.483±0.030	20.033±0.191	-	22.817±0.236	-	24.067±0.499	-	26.000±0.249	-
Std.	18.517±0.040	21.100±0.195	0.18 ↑	24.850±0.82	8.91↑	21.350±0.316*↓	11.28↓	17.883±0.149***↓	31.21↓
A	18.517±0.079	21.150±0.366	0.18 ↑	25.133±0.808	10.15↑	24.367±0.724	1.24↑	20.133±0.0211***↓	22.56↓
B	18.400±0.035	21.233±0.42*	0.44 ↑	25.483±1.039	11.68↑	24.933±0.964	3.59↑	24.300±0.23***↓	6.53↓

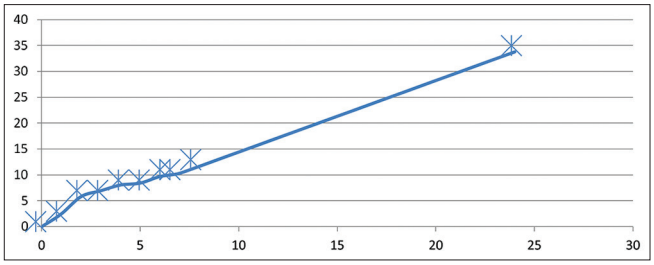

Figure 6: Cumulative percentage release of gallic acid from Hydro-alcoholic extract

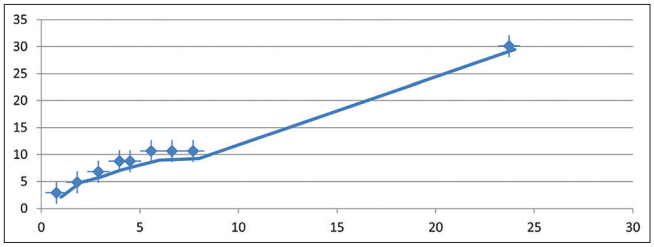

Figure 7: Cumulative percentage release of gallic acid from aqueous extract

on carrageenan-induced paw edema in Wistar rats. At the starting 4 h in which paw volume was measured statistically non-significant, inhibition of paw edema was observed in Group A and B dose-treated group in comparison to standard group. At 6th h, there is marked decrease in paw volume in standard group (11.28%), while there is no inhibition in paw volume in A and B groups. At the last 8th h, there is a significant decrease in inflammation in the standard group (31.21%) and Group A, i.e. hydroalcoholic group (22.56%), while in Group B, there was non-significant inhibition, i.e. 6.53%. In comparison between Group A and B, A showed the significant decrease in inflammation, while B group showed only mild inhibition.

CONCLUSION

It can be concluded from the results obtained that hydroalcoholic extracts could be served or can be modified for transdermal drug delivery system. As there is a less percentage of drug release *in vitro* drug release study and showing less anti-inflammatory effect so, there is a need to add permeation enhancer in the extract to increase the release of drug so that more amount of drug release from topical or transdermal dosage form.

REFERENCES

1. Anonymus. The Ayurvedic Formulary of India. Part-I. New Delhi: Department of AYUSH, MH&FW, GOI; 2003.
2. Shaker DS, El Nabarawiet MA, Attia DA, Hamed SA. *In-vitro* skin permeation and biological evaluation of lornoxicam monolithic Transdermal patches. Int J Pharm Pharm Sci 2013;5:242-8.
3. Suksaeree J, Charoenchai L, Madaka F, Monton C, Sakunpak A, Charoonratana T, *et al*. *Zingiber cassumunar* blended patches for skin application: Formulation, physicochemical properties, and *in vitro* studies. Asian J Pharm Sci 2015;10:341-9.
4. Paget GE, Barnes JM. In: Lawranle DR, Bacharch AL, editors. Evaluation of Drug Activities, Pharmacometrics. Vol. 1. New York: Academic Press; 1969.
5. Winter CA, Risely EA, Nuss CW. Carrageenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962;11:544-7.

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