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Design and development of a poly- herbal spray formulation and its physicochemical and biological profiling: from classic to modern drug delivery system

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Many of the polyherbal formulations are described in ayurvedic classics for inflammation and pains. Among them rasna saptak kwath gives best therapeutic effect in clinical studies but palatable issue makes it less popular among patients. The present work emphasizes on the transformation of this classical formulation into more convenient and acceptable form for the patients without disturbing its efficacy. In the present study kwath was converted into mechanical spray form for pain and inflammation. The extractions of herbs were done by both classical and modern methods. The mechanical spray solution was prepared with permeation enhancer, humectant, in suitable solvent system. The spray was standardized on various parameters like viscosity, evaporation time, and spray pattern. Spray was also evaluated for *in vitro* drug release, anti inflammatory, analgesic and skin irritation study. In the result, the spray pattern was found uniform and evaporation time was 9.81±0.30 min. The fluxes for spray were found to be 2.82±.0.11 (µg/cm^2/h). The kinetic model for spray best fitted to zero order permeation at a constant flux for spray r>0.98. The spray also provides good results in pain and inflammation. So, here is the possibility of replacing that sticky oil of *Ayurveda* with spray for good relief. The products show the path for a new generation of *Ayurvedic dosage* form, which has the bright future in this busy schedule of life where integrity of *Ayurvedic medicines* not hampered.

Keywords: *In vitro* drug release, Kinetic model, Mechanical spray, Permeation enhancer, Rasna saptak kwath

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Rasna saptak kwath (RSK), is scripted in many *Ayurvedic* classics like, *Bhaishajya Ratnavali*[1]*, and Sharangdhar samhita*[2]. *RSK* which is given to patients in decoction form is one such formulation, which possesses the pharmacological activities to treat symptoms of the arthritis like pain and inflammation[3]. *RSK* is a quality remedy to treat different kinds of *Amavata like Jangha* (calves), *Kati* (waist), *Pristha* (back), *Ura* (thighs) and other severe *Amavata* (Rheumatism) symptoms[1]. This formulation is composed of seven drugs. The *Sunthi* (*Zingiber officinale* Rosc.) powder is advised to be taken with decoction as an adjvant (Table 1)[2]. The ingredients of *RSK* are reported for analgesic (*Eranda* and *Devdaru*), anti-inflammatory (*Sunthi*, *Aragvadha* and *Guduchi*) and anti-arthritic activity (*Rasna* and *Gokdhura*)[4]. In Ayurvedic classics, these herbs are categorized under *sothhara* (*Rasna*), *vednasthapana* (*Gokshura*), *amavataghan* (*Guduchi*), *vatashamana* (*Eranda*)[4]. All these properties together provide good therapeutic effect in management of *Amavata* (Rheumatism) when taken in decoction form. In spite of the best results it is not acceptable by various age groups like children and old age people because of its palatability issue. Therefore patients need this unique formulation into alternative dosage form which serve as a ready to use formulation and can be used instantly to get relief from acute pain and inflammation caused by joint disorders or minor strains, sprains and contusions.

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In this research study an attempt was made to prepare a non-pressurized mechanical spray where *Rasna Saptak kwath* was converted into spray solution for topical application. The non-pressurized spray is the combine liquid mixture of the therapeutic drugs and excipients which are dissolved in defined solvent system. This whole liquid mixture is then filled in spraying bottle. The dispensing system from non-pressurized spraying bottle is based upon mechanical energy and this energy provided depression of actuator. Dip tube helps to dispense right composition of drug through metering chamber. To deliver the precise amount of therapeutic drugs from liquid mixture is based upon the knowledge of active ingredient present within the composition[5]. Maximum amount of drug reaches to the site of action through this spray which increases patient compliance and provide ease of application.

In the present study the mechanical spray of *RSK* was designed and developed with the help of some co-solvents and permeation enhancer and evaluated for its physicochemical and biological profiling *i.e.*, for pain and inflammation related to joint disorders or muscles.

Material and Methods

Procurement and identification

The raw drugs were bought from the local market Gola deena nath, Varanasi and few drugs were collected from the local area those drugs were shade dried. Plant materials were authenticated by the experts of the field.

Chemicals required

Menthol, ethyl alcohol and distilled water were purchased from science house, Varanasi, India, Dimethyl sulphoxide (Gift sample from the Department of pharmacology, IMS, BHU)

Preparation of herbal extract

The extractives were prepared by two methods one by classical method and other by contemporary method in two different medias. The aqueous extract by classical method and hydro-alcoholic extract by soxhlet method. The aqueous extractive is made to follow classical method and hydro-alcoholic media to fulfill current scenario, as today most of the pharmaceutical companies work on the hydro-alcoholic media.

Method of preparation of spray solution

The spray solution consisted of co-solvents and a permeation enhancer. All ingredients, except for the extract, were combined in a solvent system of ethanol and water at room temperature, using a magnetic stirrer set to 500 rpm for thorough mixing. Once a uniform solution was achieved, the extract was added (Table 2) and stirred at the same speed until it became homogeneous. Finally, the solution volume was adjusted by adding more ethanol to ensure the desired drug release with each spray, and the mixture was then transferred into a spray bottle.

Physicochemical evaluation

The prepared batches were optimized on following parameters[6] for standardization of herbal spray.

pH value

The pH of the formulations was determined using a digital pH meter

Viscosity

Viscosity of mechanical spray was measured with a Ostwald Viscometer.

Table 1 — Ingredients of *Rasna Saptak kwath*

S. no	Plant name	Scientific name	Part used	Amount taken (g)
1	*Rasna*	*Pluchea lanceolata* Clarke	Leaf	25
2	*Gokshura*	*Tribulus terristris* Linn.	Fruit	25
3	*Guduchi*	*Tinospora cordifolia* (Wild) Miers	Stem	25
4	*Punarnava*	*Boerhavia diffusa* Linn.	Root	25
5	*Eranda*	*Ricinus communis* Linn.	Root	25
6	*Devdaru*	*Cedrus deodara* (Roxb.) Loud	Stem	25
7	*Aragvadha*	*Cassia fistula* Linn.	Fruit	25
8	*Sunthi*	*Zingiber officinale* Rosc.	Rhizome (dry)	25

Table 2 — Composition and formulation of spray solution

S.no	Ethanol (mL)	Water (mL)	Glycerine % (v/v)	DMSO (mL) % (v/v)	Menthol (mL) % (v/v)	DRUG (mg)
S1	4	6	15%	20%	5%	100
S2	5	5	10%	20%	5%	100
S3	6	4	5%	20%	5%	100

The solution was introduced into the viscometer and with the help of suction pulled into the upper reservoir and then drained into the lower reservoir by gravity. The time that it takes for the liquid to pass between two etched marks, one above and one bellow the upper reservoir, is measured. The relative viscosity is:

$\eta/ \eta 0 = \rho\, t/ \rho 0\, t0$

Where, ρ is the density, t is the time of outflow of the sample, $\rho 0$ is the density, t0 is the time of the outflow of the reference liquid (water). Knowing $\eta 0$ the viscosity of the sample can be calculated.

Evaporation time

Evaporation time is the time required to evaporate the solvents after spraying the formulation and leaving behind only therapeutic drug. The drying time was noted after spraying the formulation on white paper at an average room of temperature 32ºC- 34ºC.

Spray pattern

Spray pattern was obtained by spraying the liquid on light color paper. The formulation was sprayed on a fixed white paper from the distance of 2-3 cm and diameters and the spraying pattern of the spots were measured.

Average weight per dose

After taking the initial weight of the spray bottle with formulation the formulation was sprayed from the containers. After the five successive deliveries the spray bottle was weighed for final weight.

Average weight per dose =

Initial weight - Final weight/Number of deliveries

Biological activity evaluation

The experimental protocols were approved by Institutional Animal Ethics Committee Institute of Medical Sciences, Banaras Hindu University, Varanasi (IAEC No. 542/02/ab/CPCSEA 27.12.2012) in accordance with the guideline formulated by CPCSE, India.

In vitro drug release of final product

This study was performed[7] with the aim to know the percentage of drug released from the spray solution. Gallic acid was present in herbal extract and it is also taken as marker compound.

Preparation of rat skin

The male albino rats weighing (150-200 g) were selected and their abdominal hairs removed by Veet®. After the animals were sacrificed, the abdominal skin with full thickness was removed. Isopropyl alcohol is used for removing any traces of fat present on skin surface. Skin was rinsed with distilled water and stored at- 20°C soaked in saline.

In vitro transdermal permeation study

Franz diffusion cell was used to conduct *in vitro* diffusion study. The two compartments of Franz diffusion cell, the donor and receptor separated by rat skin. The stratum corneum of the skin should face towards the donor compartment in which the spray formulation of 5 mL was filled. The receptor compartment was filled with 25 mL of phosphate buffer of pH 7.4. A thermostatic bath was used to maintain the temperature at 32 ± 0.5°C of the receptor compartment and 600 rpm rotation speed was set while performing the experiment. At predetermined time intervals the 5 mL of receptor medium were withdrawn and simultaneously 5 mL of fresh phosphate buffer of pH 7.4 introduced into the receptor medium. The amount of gallic acid permeated through the skin in receptor medium were calculated through UV spectrophotometer at 261 nm. The permeation study was conducted for 24 h.

Evaluation of test drug effect on carrageenan induced paw oedema in rats

The anti inflammatory effect of the test drug was studied on rats through Carrageenan induced paw oedema method. The inflammation was induced into sub plantar tissues of the left hind paw of each rat by injecting the suspension into sub plantar tissues of the left hind paw[8]. The suspension was prepared by 0.1 mL of 1% w/v carrageenan suspended in 1% CMC. *Charles Foster* albino rats of either sex weighing between 150-250 g were selected for the experimentation. The paw volume was measured, and the oedema volume and Inhibition rate were calculated using following formula.

Oedema volume = $Vt - Vc$

Where, Vt is paw volume in mL, at time t, after carrageenan administration. Vc is paw volume in mL, before carrageenan administration.

$$\text{Inhibition rate (\%)} = \frac{Ec - Et}{Ec} \times 100$$

Where, Ec = oedema volume of control group and Et = oedema volume of treated group.

Evaluation of test drug effect on acetic acid induced writhing in mice

Swiss albino mice of either sex were used for the study. Numbers of writhing (W) were counted for 20 min after exclusion of the first 5 min[9]. The pain inhibition ratio (PIR) was calculated for each group using:

$$PIR\% = \frac{Wc - Wt}{Wc} \times 100$$

Where Wc = writhing numbers of the control group
Wt = writhing numbers of the test groups

A) Skin irritation study

The RS spray was evaluated for any oedema and erythema on animal skin after their application. Swiss albino mice were separated into two groups each having 6 mice. The study period was conducted for 7 days and each mouse was treated once daily. The groups were as follows:

Group I- 0.8% v/v aqueous Formalin solution,

Group II- 0.8% v/v aqueous Formalin solution + RS Spray

The application site was visually evaluated for erythema and oedema on the 8th day[10].

Statistical data analysis

For all the studies, the data were calculated and presented as Mean ± SD (n=6). Statistical analyses of the data were performed using One Way ANOVA followed by Dunnett's for between the group comparisons. Paired t-test was applied for within the group comparison. The level of significance was taken as $p \leq 0.05$.

Statistical significance

$p<0.05^{*}$ was considered as statistically significant $p<0.01^{**}$ and $p<0.001^{***}$ as statistically highly significant $p>0.05^{\#}$ as not statistically significant.

Results

The extraction of seven herbs of *Rasna Saptak* formulation was carried out through two different processes**.** The percentage yield was more in hydro-alcoholic extraction i.e. 12.4±0.78 than AQ extract *i.e.*, 10.1±0.3[9]. The prepared preliminary batches were tested on different parameters (Table 3). Batch S1 and S2 showed non uniformity in spray pattern while S3 batch showed uniform spray pattern and less evaporation time. So, S3 batch was selected for final studies. The evaluation of final batch (S3) which was formed in triplicate was done (Table 4). The pH and evaporation time were 1.3±0.06 and 9.81±0.30 respectively. Viscosity and average wt. /dose were 15.6±0.15 and 0.40±0.19 respectively.

In vitro drug release study, 8 mg of gallic acid was present in 5 mL of spray solution[9]. The spray solution exhibited 67.08±2.29% (Fig. 1 and Table 5) of drug permeated in 24 h. with flux of 2.82±.0.11 µg/cm² /h and with a permeation coefficient of 0.35±0.0146 cm^2/h. The regression equation for different kinetic model was applied for final spray solution. The highest regression (R^2) was found for zero order *i.e.,* 0.98 (Fig. 1).

The magnitude of suppression in carrageen an induced paw swelling (Table 6 and Table 7) was 43.3% for *RS* spray. RS spray inhibited acetic acid writhing up to 37.73% (Table 8). *RS* spray showed little erythema and negligible oedema and no sensitivity or irritation type of reaction caused by polyherbal spray formulation (Table 9).

Discussion

Development of a poly- herbal spray formulation

The synergistic effect of water and alcohol with the assist of heat in soxhlet extraction is responsible for

Table 3 — Optimization of preliminary batches of mechanical spray

Batch	Evaporation Time (Min.)	Viscosity (cps) (mm^2/s)	pH	Spray pattern
S1	15.6	23.6	6.5	Non-uniform & longitudinal
S2	13.0	20.1	6.7	Non-uniform & longitudinal
S3	9.2	16.5	5.4	Uniform & spherical

(n=3)

Table 4 — Evaluation parameters for final Spray

S. no	Parameters	Mean ± SD
1	pH	1.3±0.06
2	Evaporation time (minutes)	9.81±0.30
3	Spray Pattern	Spherical and uniform
4	Viscosity (mm²/s) (centipose)	15.6±0.15
5	Average wt per dose (%) (w/w)	0.40±0.19

(Mean± SD; n=3)

Table 5 — Result for *in vitro* drug release (Gallic acid) of mechanical spray

Spray	CAR (mg) (24th h.)	CPR % (w/w) (24th h.)	Flux (Js) (ug/cm^2/h)	Kp (cm^2/h)
S3	5.36±0.183	67.08±2.29	2.82±.0.11	0.35±0.0146

(Mean±SD, n=3; CAR-Cumulative amount release; CPR-Cumulative percentage release; Kp-Permeability coefficient)

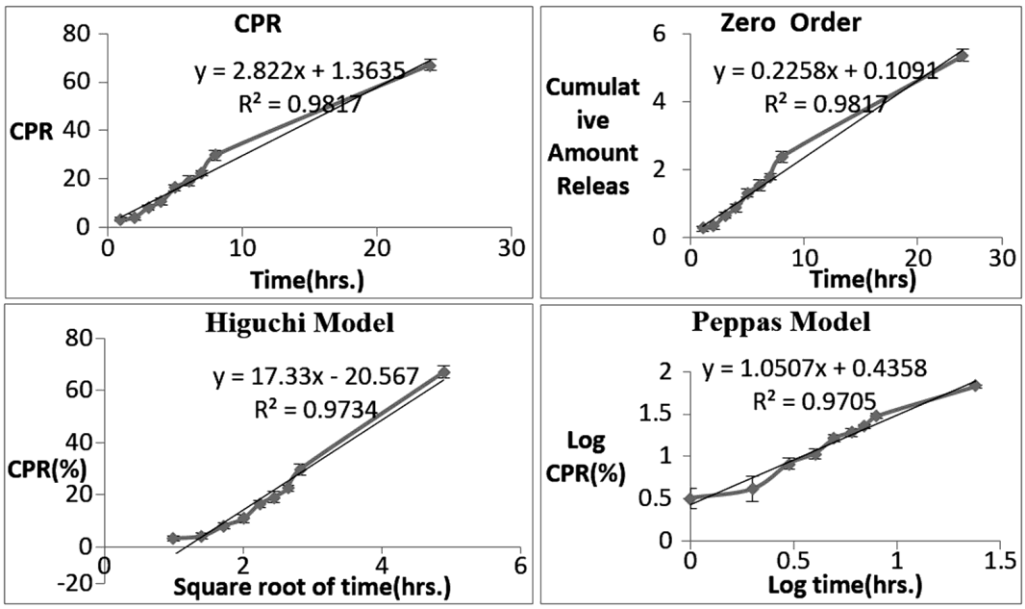

Fig. 1 — *In vitro* drug release profile of final spray solution (Mean ±SD; n=3)

Table 6 — Difference in swelling score (mL) between the hours. Mean± SD

Groups	H 0-h 2	H 0-h 4	H 0-h 6	H 0-h 8
Carragennan Control	3.61±0.13 t=66.651 p<0.001	4.28±0.11 t=89.748 p<0.001	3.51±0.11 t=73.684 p<0.001	2.98±0.14 t=49.649 p<0.001
RS Spray	3.53±0.10 t=83.800 p<0.001	3.95±0.16 t=58.883 p<0.001	2.35±0.15 t=37.956 p<0.001	1.68±0.14 t=28.012 p<0.001

(Within the Group comparison (Paired "t" test)) (n=6) $p<0.05^{*}$ was considered as statistically significant; $p<0.01^{**}$ and $p<0.001^{***}$ as statistically highly significant; $p>0.05^{\#}$ as not statistically significant)

Table 7 — Percentage inhibition of paw oedema (mL)

Treatment Groups	2nd h	4th h	6th h	8th h
RS spray	2.7%	9.3%	34.6%	43.3%

Table 8 — Result for analgesic activity

Groups	Dose	WRF(Mean + SD)	PIR
Control	----	53.16±.047	-----
RS Spray	2.5 mL	33.5±0.34	37.73%

(Mean + SD; n=6: WRF- Writhing Frequency; PIR-Percentage Inhibition Rate)

Table 9 — Result for skin irritation study

Rat no.	Group I		Group II	
	Erythema	Oedema	Erythema	Oedema
1	3	3	0	0
2	3	3	0	0
3	3	3	4	1
4	3	2	1	0
5	2	3	0	1
6	3	3	0	0
Mean±SD	2.66±0.216	2.5±0.21	0.83±0.196	0.33±0.17

(n=6; Erythema and Oedema scale: 0-none;1- slight; 2- well defined; 3- moderate; 4- scars formation/severe)

the mass transfer of metabolites into the solvent[11]. The various factors on which maximum extraction depends are polarity of the solvent, kinetics of mass transfer and rate of mass diffusion etc.[12]. The designing of system depends upon the physical and chemical properties of drugs used[13]. The solvent system for the spray was the mixture of aqueous and non-aqueous solvents. The final volume of the spray was composed of a non-aqueous solution, as higher concentrations of aqueous components could promote microbial growth. Ethanol serves as a preservative, reducing the risk of such growth. The spray solution contained various chemicals and extracts that could produce an unpleasant odor for patients. To address this, 5% menthol was added to provide a more pleasant fragrance and a cooling sensation on the skin. Additionally, glycerin was incorporated into the co-solvents at concentrations ranging from 5% to 15%, which helps retain the drug particles after the co-solvents evaporate, thereby increasing the interaction time between the drug and the skin. Humectant retains the moisture through absoprtion[14]. For more permeability of the drug across the skin a permeation enhancer was also added for which dimethyl sulphoxide (DMSO) was chosen.

Physicochemical profiling

The three different batches were evaluated for different physical parameters *viz.*, viscosity, pH and spray pattern. The viscosity of batch S1 was 23.6 cps which is quite high. Viscosity reflects the fluidity of spray; it should not be too thin and not be too viscous. The batch S3 showed 16.5 cps viscosity. The S1 and S2 batch showed almost same pH, which suggest that both the batch would not be compatible with the skin because skin pH is 4 to 5 and if pH is not compatible with skin then it may produce irritation or any adverse effect on skin. The ph of S3 is 5.4 and is compatible with the skin. The evaporation time is the time taken by the co-solvent to evaporate from skin and leaving behind the drug with humectants over the skin. From the (Table 3) it was revealed that in preliminary batch evaporation time varies from 9 to 15 min. S1 batch showed highest time for evaporation. The highest percentage of glycerin is probably responsible for these numbers. Even the spray pattern of both the batch S1 and S2 were not uniform. The high viscous solution resist the uniform spraying of solution and the nozzle size may be secondary reason for non-uniform and longitudinal spray pattern. Spray pattern should be uniform to cover equal and maximum area. The S3 batch showed low viscosity, minimum evaporation time and most important suitable pH *i.e.*, 5.4. Even the spray pattern was also uniform and spherical. The low viscous solution might be responsible for uniform spraying, so role of nozzle size and shape responsible for spraying pattern is questionable. Finally from the above analysis S3 batch was selected for biological profiling of mechanical spray. Average weight per dose confirms its uniformity and it was found that final batch showed satisfactory result for all parameters. It confirms the process is validated for making mechanical spray.

Biological profiling

In vitro skin permeation study of the topical spray solution S3 exhibited 67.08±2.29% of drug permeation in 24 h (Fig. 1). The result is due to the permeation enhancer DMSO which played its role and increases the permeation of drug across the skin. DMSO acts as a vehicle for topical drugs and smoothes the progress for the transport of drug molecules into the horny layer of the skin. It helps to transport non – ionized molecule of low molecular weight and it is also has the ability to change the alpha helix intercellular keratin confirmation to a beta sheet[15]. Many studies revealed that DMSO enhance the permeation effect of both hydrophilic and lipophilic substances[16]. The number of molecules penetrating through a given cross-sectional area during a given period of time is known as Flux. The drug permeated with a flux of 2.82±.0.11 μg/cm² /h and with a permeation coefficient of 0.35±0.0146 cm^2/h (Fig. 1). The quantitative measure of the rate at which a molecule can cross a membrane is known as permeability coefficient[17]. The flux and coefficient both indicates that the permeability of molecules of the drug across the skin is low and that is why only 67% drug passes through the skin in 24 h. The large molecules might be responsible for this effect. As large molecules had blocked the skin pores which hindered the permeability of small molecule drugs. To know the drug release kinetics model of optimized formulation (Batch S3) zero order, Higuchi and Peppas model were constructed. It was found that optimized batch was better fit to a zero order (R^2=0. 981), than Higuchi (R^2=0.979), and Peppas (R^2=0.978) model. MS follow fickian diffusion, where the rate of drug release is independent of the drug concentration *i.e.*, zero order. It indicates that there will be reduction in the frequency of drug administration[18].

In animal studies the mechanical spray (MS) showed noted inhibition of inflammation and pain. The study for inflammation was carried out for 8 h only. The major drawback in animal study was the wiping off the spray from animal paw due to which the contact time of spray with animal paw was not constant throughout the study. The inflammation and pain was reduced up to 43% at 8^{th} hr and 37% respectively in comparison to control group where no medicament was provided. Probably the phytoconstituents like phenol, alkaloids, and saponins act on the inflammatory mediators or inhibits cycoloxygenase and lipooxygensae which result in anti inflammatory and analgesic activity of spray[19]. If contact time of spray with body is increases then the anti-inflammatory effect may also increase. The skin irritation study done on animal skin showed that the formulation does not produce irritation to the skin and hence supposed to be safe for human use also.

From the above discussion it signifies that spray is capable to provide the relief from joint disorder or muscular pain and inflammation at moderate level when no other medicament was provided.

Conclusion

Through the present experimentation, it has been found that the *RSK* formulation of ayurvedic origin can also be utilized as topical application by incorporating various chemicals like DMSO as permeation enhancer. The physicochemical and biological evaluation shows that the results are under limit which is necessary for the formulation for its effective use and it is safe for moderate pain and inflammation caused by joint disorders or muscles. However, a research study between RSK and MS is suggested to know their comparative therapeutic action in joint disorders. This experimentation is one of the first few attempts to utilize ayurvedic formulation into other convenient modern dosage form. It can be concluded that MS, is a rational alternative to oral *RSK* for the treatment of inflammatory soft tissue conditions. It is an optional and acceptable formulation of *RSK* which is easy to carry, apply, and comfortable to use.

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Conflict of Interest

No conflict of interest exists.

Author Contributions

AKC proposed the formulation and ideas for novel formulation; SP conceived the presented idea and performed the pharmaceutical analytical study. SM contributed in experimental study. BM provided chemicals and lab facilities and valuable suggestions while performing research. The original manuscript was edited and reviewed by SP. The findings were discussed by all authors and they all contributed to the final manuscript.

Data Availability

Author will provide the data on reasonable request.

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