

Effect of Nanosizing on Bio-efficacy of *Shwaskuthar Rasa* - A Herbomineral Formulation for Asthma and Allergy

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Abstract:

To assess the effect of nanosizing on bio-efficacy of *Shwaskuthar Rasa* - a herbomineral formulation in albino rats for asthma and allergy. *Shwaskuthar Rasa* - a renowned traditional herbomineral formulation of Ayurveda was nanosized by planetary micromill (P-7) and its particle size was characterized by dynamic light scattering method. The antiasthma and antiallergic activity of nanosized *Shwaskuthar Rasa* was evaluated in albino rats by clonidine induced mast cell degranulation and catalepsy. There was significant percentage protection of mast cell (44.13%) found in the SWR72 treated group compared to control group (27.16%) and significant inhibition in the catalepsy over the control group. Size reduction shows that *Shwaskuthar Rasa* was significant protection on mast cell degranulation and catalepsy in rats which is fineness dependant i.e. smaller the particle size, better the antiasthmatic and antiallergic effect.

Key Words: Catalepsy, Particle size, Mast cell, *Shwaskuthar Rasa*

Introduction:

“Rasa Shastra” although basically means the “science of mercury” but also refers to the science of making minerals suitable for the body so that they can be used as medicines^[1, 2]. Minerals such as mercury and arsenic are considered toxic^[3] but by proper shodhana (detoxification), they can be made into wonderful medicines. When mercury is properly prepared, it balances all three *doshas* (humours) of the body, has a soothing effect and prevents disease and aging process. It nourishes all vital body parts and increases the strength of the eyes^[4, 5]. It is *rasayana* (rejuvenating), *vrana sodhana*, ropana (wound cleaner and wound healer) and *krimighna* (anthelmintic and antimicrobial)^[6]. When compounded with any herb the mercury heightens the medicinal properties of the herb. Mercury is said to give a firm physique, a stable mind, and considered to be the destroyer of diseases. When used in the combination or along with rasa, the efficacy spectrum of herbs increases to a great extent enabling them to treat complicated diseases. It is for this reason that *Rasa Shastra* has been placed with so much importance and high esteem in Ayurveda.

Shwaskuthar Rasa a highly valued herbomineral formulation of Ayurveda for the treatment of asthma and allergy

contains herbs pure aconite, pippali, black pepper, ginger and minerals pure mercury, sulphur, borax and arsenic (As_2S_2) formulated as per Ayurvedic text^[7]. Review of literature revealed that *Shwaskuthar Rasa*, apart for treating asthma, allergy, is used in the treatment of cough, laryngitis, tuberculosis, unconsciousness, mental disorders, comma, chest burn and heart disease etc^[7]. The present study aims to open new vistas for establishing the effect of nanosizing on *Shwaskuthar Rasa* for enhancing its bio-efficacy for treating allergy, asthma and other respiratory problems. As surface area increases due to size reduction, the therapeutic efficacy of formulation increases due to enhanced release, dissolution and bioavailability, the nanosizing of *Shwaskuthar Rasa* formulation therefore might improve its effectiveness and hence the study was undertaken.

Materials and Methods:

Instruments

For nanosizing the market available *Shwaskuthar Rasa* tablets, planetary micromill (Fritsch GmbH Model-P7, Germany) providing high speed grinding of laboratory samples down to $< 1 \mu m$ and suitable for grinding hard to soft materials was used. The particle size reduction of *Shwaskuthar Rasa* was assessed by dynamic light scattering method.

Preparation and measurement of nanoparticle

Hundred tablets of *Shwaskuthar Rasa* procured from the Dabur Research Foundation were crushed and pulverized using pestle and mortar for 2 hour and the powder was passed through 300 mesh sieve and powder so obtained was microscopically examined using calibrated micrometer eyepiece for particle size and morphology. The shape of the particles was found to be irregular and particle size 10 to 100 μ m was grossly ununiform. Overall 250 particles were measured along an arbitrary fixed line and median diameter was calculated [8]. The median diameter is the diameter for which 50% of the particles measured are less than the stated size (Figure 1).

Powder (10gm) of *Shwaskuthar Rasa* obtained from passing to 300 mesh sieve having average particle size of 47.61 μ m was placed in the 45ml bowls of planetary micromill and screwed tightly. The micromill was set on 300 rpm and samples were collected after 6, 12, 18 and 24hr respectively. The rotation direction of micromill was reversed after each 15 minutes to prevent sticking of material on the inner wall of the bowls. The samples of *Shwaskuthar Rasa* so collected after fixed time intervals were assessed for particle size by dynamic light scattering method (Table 1).

Experimental animals

Albino rats of either sex weighing 120-150g were kept in colony cages at ambient temperature of $28^{\circ} \pm 2^{\circ}$ C and 45 to 55% relative humidity with a 12 hours light/dark cycle. They were allowed free access to standard diet and water *ad libitum*. The protocol for animal experimentation approved by IAEC (Dr. H. S. Gour Central University, Sagar) was followed.

Mast cell degranulation in rats

Albino rats were divided in six groups of six animals each and a three day drug treatment schedule was followed. Group-I animals received 2% w/v acacia

suspension (10ml/kg, p.o). Group-II animals were treated with disodium chromoglycate (50 mg/kg, i.p.) as standard drug, whereas animals of Group-III, IV, V and VI were treated with a suspension of different particle size of *Shwaskuthar Rasa* (23mg/kg, p.o.) for three days. On the fourth day, the rats were anaesthetized with ether and each animal was injected with 4ml/kg of saline solution (0.9% w/v) into peritoneal cavity and the abdomen was gently massaged for 90 sec. The peritoneal cavity was carefully opened, and fluid containing peritoneal mast cells was aspirated, collected and transferred into test tubes containing 7-10 ml RPMI-1640 buffer medium (pH 7.2-7.4). This solution was then centrifuged at 400-500 rpm to get mast cells. Pellet of mast cells was washed with same buffer medium twice by centrifugation. Discarding supernatant, cells were challenged with clonidine (50 μ g), incubated (37 $^{\circ}$ C) in a water bath (10 minutes), stained with 1% Toluidine blue and observed under microscope (45X). Total 100 cells were counted from different visual area [9, 10]. Percentage protection against degranulation of mast cells was calculated in all groups i.e. percentage of protected mast cells among total 100 cells was calculated (Table 2).

Clonidine induced catalepsy in rats

Albino rats were divided in six groups consisting of six animals per group. Group-I animals received 2% w/v acacia suspension (10ml/kg, p.o) served as control. Group-II received standard drug Pheniramine maleate (10mg/kg i.p) and animals of Group-III, IV, V and VI were administered different nanosized *Shwaskuthar Rasa* (23 mg/kg, p.o) for three days. After one hour of last dose, clonidine (1mg/kg, s.c.) was injected to all groups of animals. The forepaws of rats were placed on a horizontal bar (3 cm in diameter, 6 cm above the table) and the time required to remove the paws from bar was noted for each animal [11].

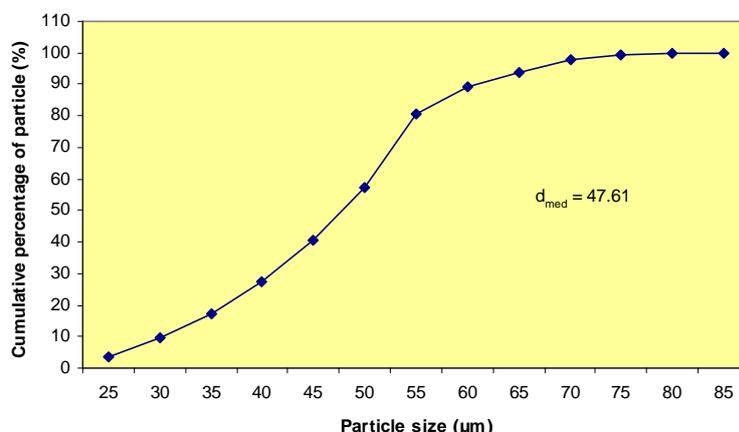


Figure 1: Cumulative distribution plot for determination of the median size of simple pestle-mortar pulverized *Shwaskuthar Rasa*.

Table 1: Particle size of *Shwaskuthar Rasa* obtained from planetary micromil after different time intervals.

S.No	Time interval (hr)	Average particle size (nm)	Sample nomenclature
1.	6	744	SWR 744
2.	12	289	SWR 289
3.	18	136	SWR 136
4.	24	72	SWR 72

Table 2: Effect of different particle size of *Shwaskuthar Rasa* on clonidine induced mast cell degranulation in rats

Group	Treatment	Dose (per kg) p.o	Percentage mast cell protection (Mean \pm SEM)
I	Vehicle control	10ml	27.16 \pm 1.70
II	Disodium chromoglycate	50mg (i.p)	77.18 \pm 2.34**
III	SWR (744)	3.5mg	33.45 \pm 1.31
IV	SWR (289)	3.5mg	37.26 \pm 1.55*
V	SWR (136)	3.5mg	41.20 \pm 1.24*
VI	SWR (72)	3.5 mg	44.13 \pm 1.36**

**= p<0.01 considered more significant as compared to vehicle control.

*= p<0.05 considered significant as compared to vehicle control.

Duration of catalepsy was measured at 0, 15, 30, 60, 90, 120, 150 and 180 minute of interval (Table 3).

Statistical analysis

All values were expressed as mean \pm SEM. Statistical significance was calculated using one-way ANOVA followed by Dunnet comparison test. P values < 0.05 were considered significant.

Results and Discussion:

Shwaskuthar Rasa, a reputed herbomineral formulation of Ayurveda, apart from many therapeutic potential is specially valued for respiratory problems with specific effect on asthma and allergy. *Shwaskuthar Rasa* tablets were pulverized using pestle mortar followed by planetary micromill. Subsequently samples were withdrawn from micromill at intervals of 6, 12, 18 and 24 hrs and

Table 3: Effect of different particle size of *Shwaskuthar Rasa* on clonidine induced catalepsy in rats

Treatment (per kg body Wt. p.o.)		Duration of Catalepsy (in sec) Mean \pm SEM							
		0 min	15 min	30 min	60 min	90 min	120 min	150 min	180 min
Group-I (Control)	10ml	12.5	47.5	88.3	104.5	159.2	104.2	70.33	38.3
		\pm 2.22	\pm 4.05	\pm 3.12	\pm 3.57	\pm 3.69	\pm 2.56	\pm 2.34	\pm 2.52
Group-II (Phenirami ne maleate)	10mg/ kg, i.p.	12.1	26.0	61.2	69.6	112.1	63.2	36.4	14.0
		\pm 0.46	\pm 2.38	\pm 3.14	\pm 3.58	\pm 2.32	\pm 2.89	\pm 1.64	\pm 0.92
Group-III SWR ₇₄₄	3.5mg	14.2	40.3	80.5	95.1	136.5	87.0	57.8	32.6
		\pm 1.77	\pm 3.02	\pm 2.37	\pm 1.97	\pm 3.54*	\pm 2.83*	\pm 3.60*	\pm 2.37
Group-IV SWR ₂₈₉	3.5mg	11.8	37.6	74.8	86.5	132.5	80.5	53.1	28.3
		\pm 0.60	\pm 2.37	\pm 3.60*	\pm 3.83	\pm 2.45*	\pm 2.89**	\pm 1.66**	\pm 2.49*
Group-V SWR ₁₃₆	3.5 mg	12.2	34.8	70.5	84.6	123.5	74.0	49.1	25.2
		\pm 1.22	\pm 1.46*	\pm 2.81**	\pm 2.81*	\pm 2.88**	\pm 1.50**	\pm 2.45**	\pm 1.46**
Group-VI SWR ₇₂	3.5mg	11.4	29.0	65.6	78.5	117.5	65.6	40.3	20.8
		\pm 0.80	\pm 3.51*	\pm 2.31**	\pm 3.23**	\pm 2.78**	\pm 3.05**	\pm 2.96**	\pm 1.24**

**= p<0.01 considered more significant as compared to control, *= p<0.05 considered significant as compared to control.

measured for their particles size, where average particles size of samples was found to be 744 nm, 289 nm, 136 nm and 72 nm respectively.

For mast cell degranulation method, the study revealed that there is correlation of particle size of *Shwaskuthar Rasa* formulation with the mast cell degranulation and the release of histamine after administration of mast cell degranulating agent. The standard drug disodium chromoglycate (50 mg/kg, i.p) showed a maximum of 77.18% protection of mast cells. Among the *Shwaskuthar Rasa* treated groups the maximum percentage protection of mast cells 44.13% was found in the SWR72 group compared to control group (27.16%). This was followed by 41.20% and 37.26% with SWR136 and SWR289 respectively and lowest protection 33.45% was found with the SWR744 over the control group. The mast cell degranulation and histamine release

study revealed that the effect of nanosized *Shwaskuthar Rasa* is fineness dependant i.e. particle size dependent.

Catalepsy is a condition in which the animal maintains imposed posture for longtime before regaining normal posture. Catalepsy is a sign of extrapyramidal effect of drugs that inhibit dopaminergic transmission or increase histamine release in brain. Several drugs are known to induce catalepsy in animals, α_2 -adrenoceptor agonist induce catalepsy by inhibiting dopamine D2 receptors in the substantia nigra. Different stages of catalepsy appear to be directly correlated with brain histamine content^[12]. To study the effect of nanosizing *Shwaskuthar Rasa* on clonidine induced catalepsy in rats, bar test was used. In mast cell degranulation study, the effect of *Shwaskuthar Rasa* was fineness dependant. Among the various particle sizes of *Shwaskuthar Rasa*, the SWR72

(smaller particle size) showed maximum inhibition in the catalepsy over the control group probably due to inhibiting dopamine release, whereas SWR744 (with relatively bigger particle size) showed the least inhibition in the catalepsy, therefore it can be concluded that efficacy of the *Shwaskuthar Rasa* increases as the particle size decreases facilitating the drug to cross the biological barriers thus increasing the bioavailability of the formulation which may eventually be responsible for its enhanced antihistaminic activity.

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