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Anti-tussive activity of *Shwasakuthara Rasa* a Herbo-mineral formulation prepared with and without *Kajjali* (Black Sulphide of Mercury) in SO_2 induced cough in Swiss albino mice

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ABSTRACT

Introduction: *Kajjali* is considered as the base in maximum Rasa Yogas (Herbo-mineral formulations). *Shwasakuthara Rasa* (SKR) is a well-known herbo-mineral formulation indicated in different kinds of *Shwasa* (respiratory diseases) and *Kasa* (cough) having *Kajjali* as a base ingredient. The present study is to evaluate the acute toxicity and anti-tussive activity of SKR one prepared with *Kajjali* (SKR1) and another without *Kajjali* (SKR2) in sulphur dioxide induced cough model in albino mice. **Materials and Methods:** Acute toxicity study was carried as per the OECD 425 guideline in wistar female rats. Anti-tussive activity was carried out against sulphur dioxide-induced cough reflex in mice. **Results:** Animals did not manifest any signs of toxicity and mortality at the dose of 2000mg/kg body weight, orally. Both test drugs (32.5 mg/kg, po) showed significant reduction in cough reflexes compared with control. SKR1 showed pronounced anti-tussive activity followed by SKR2 when compared to control group. **Conclusion:** The presence of *Kajjali* in the formulation is safe on acute administration and further enhances anti-tussive activity of the formulation may be due to increasing bioavailability of Ayurvedic formulation.

Keywords: Acute toxicity, Anti-tussive, Kajjali, *Shwasakuthararasa*, Sulphurdioxide(SO_2).

INTRODUCTION

Mercury is considered as the nucleus of *Rasoushadhies* (herbomineral formulations) [1]. *Kajjali* is black sulphide of mercury which is prepared from classically treated processed mercury and sulphur and used as an intermediate product in maximum rasa formulations [2]. *Kajjali* is said to possess *Rasayana*(anti-aging) and *Yogavahi* (as a catalyst) property. Addition of *Kajjali* in various herbal powders increase the shelf life and bioavailability of respective herbs [3]. There are many publications regarding the use of heavy metal content in Ayurvedic formulations. For example heavy metals in traditional Indian remedies [4]; lead poisoning from traditional Indian medicines [5]; Heavy metal content of ayurvedic herbal medicine products [6]; arsenic and mercury intoxication due to Indian ethnic remedies [7] simultaneous exposure to lead arsenic and mercury from Indian ethnic remedies etc [8]. These are the reasons now the public is afraid of using ayurvedic formulations because heavy metal content likes mercury, arsenic, and lead in Ayurvedic formulations. Thus there is an immense need to study the mercurial preparations for their safety and efficacy. In this direction the present study is planned to prepare *Shwasakuthara Rasa* (SKR) a herbo-mineral formulation with and without *Kajjali*(black sulphide of mercury)and their comparative safety and efficacy evaluations in experimental animals. Researches of recent past have proven anti-tussive activity of many herbs and poly-herbal formulations. But there are very few studies on herbo-mineral or mineral formulations for antitussive activity; one attempt was made with *Sameerapannaga Rasa* which is an arseno-mercurial formulation showed highly significant antitussive activity [9]. Most of the herbal drugs used in *Shwasakuthara Rasa* proven their antitussive, anti-inflammatory and anti-allergic activity and used more frequently in the diseases of respiratory system. Thus here an attempt was made to evaluate the acute toxicity and comparative anti-tusstive activity of two samples of SKR with and without *Kajjali* in mice with following aims and objectives to assess the safety and role of *Kajjali* in therapeutics.

MATERIALS AND METHODS

Test Drug: The trial drugsSKR1(SKR with *Kajjali*) and SKR2 (SKR without *Kajjali)*was prepared in the Departmental laboratory by following standard manufacturing procedures as explained in Ayurvedic Formulary of India [10]. The formulation compositions of 2 samples of SKR are placed at Table-1. Genuine raw materials certified by the authority were procured from Pharmacy, Gujarat Ayurved University, Jamnagar. Herbal drugs were identified in the Pharmacognosy laboratory. Processing of raw

was done as per classical methods. For SKR1 first *Kajjali* was prepared using processed *Parada* (mercury) and *Gandaka* (sulphur). To this *Kajjali*, fine powders of other ingredients like processed *Vatsanabha (Aconitumchasmanthum* Stapf.*)*, processed *Tankana* (Borax), processed *Manahshila* (Realgar), *Maricha (Pipernigrum* Linn.), *Pippali* (*Piperlongum* Linn.) and *Shunthi (Zingiberofficinale* Roscoe.) were added one by one and triturated well to get a homogenous mixture. In case of SKR2 fine powders of processed *Vatsanabha, Tankana, Manahshila, Maricha, Pippali* and *Shunti* were added one by one and triturated well to get homogenous mixture.

Table 1: Showing the formulation compositions of SKR 1and SKR2

Sr. No.	Drug Name	SKR 1	SKR 2
1	*Kajjali* (Black sulphide of Mercury)	2P	--
2	*ShuddhaVatsanabha* (Processed aconite)	1P	1P
3	*ShuddhaTankana* (Processed Borax)	1P	1P
4	*ShuddhaManashila* (Processed Realgar)	1P	1P
5	*Marichachoorna* (powder of *Pipernigrum* Linn.)	9P	9P
6	*PippaliChoorna* (powder of *Piperlongum* Linn.)	1P	1P
7	ShunthiChoorna (powder of *Zingiberofficinale* Roscoe.)	1P	1P

Animals

Wistar Strain female albino rats of either sex weighing between 200±20g and Swiss albino mice weighing between 30±5 g were used for experimental study. The animals were obtained from the animal house attached to the pharmacology laboratory of the institute. Animals were exposed to 12h light and dark cycles with ideal laboratory conditions in terms of ambient temperature (22±2ºC) and humidity (50-60%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and drinking water given *ad libitum*. The experiment was carried out after obtaining permission from Institutional Animal Ethics Committee (IAEC/19/2015/40).

Acute Toxicity

Acute oral toxicity of two samples of SKR was carried out in Wistar strain female albino rats as per the OECD 425 guidelines. The formulation was tested at the oral limit dose of 2000 mg/kg body weight in sequential manner. The result showed that both samplesdid not produce any changes in observed parameters and there is no mortality even at the limit dose of 2000 mg/kg, orally. Hence, the animal dose was fixed on the basis of human therapeutic dose mentioned in classical literature.

Anti-tussive activity

The mice were divided into four groups of six animals each.Group (I) received honey plus distilled water and served as control (5 ml/kg, oral). The test formulations SKR 1 and SKR 2(32.5 mg/kg, po) were administered to the groups (II) and (III)respectively. The mice dose was fixed extrapolating the human dose (250 mg/day) based on the body surface area ratio [11]. Test drugs were mixed with honey and suspension was prepared with distilled water and administered orally using oral canula. Standard drug Recodex (Wockhardt Ltd., Mumbai, India), containing codeine phosphate (2mg/ml) and chlorpheniramine maleate (0.8mg/ml) in the dose of 5ml/kg was administered to Group (IV). The test drugs and standards were administered to mice one hour before the sulphur dioxide exposure.

Anti-tussive effect of the test formulations was evaluated in mice by following the procedure of Miyagoshi *et al*.(1986) [12]. In brief, the assembly comprises of a 500ml three necked flask containing aqueous saturated sodium hydrogen sulphite solution ($NaHSO_3$; Nice Chemicals Pvt. Ltd.). Into this bottle, concentrated sulphuric acid was introduced drop by drop. The reaction involved is: $2NaHSO_3+H_2SO_4 = 2SO_2+Na_2SO_4+H_2O$.

SO_2 is filled previously in the column of water manometer by opening the three-way cork such that SO_2 can enter the water manometer but without any exit way until the pressure generated reads 75 mm of water as recorded by the water manometer. Then the three-way cork is rotated in such a way that the volume of SO_2 collected in the water manometer escapes into the desiccators and not into the flask containing sodium hydrogen sulphate solution. The mice to be tested is placed in the desiccator and covered with lid. Amount of SO_2 is introduced into the desiccator by this procedure. The mice, after exposure to SO_2 for one minute in the desiccators, were taken out of the desiccator and confined in an up-turned filter funnel. The free end of the funnel is attached to a stethoscope, by the help of which the cough reflex of the mice was heard and the number of cough episodes in five minutes was enumerated. To avoid the observer bias, cough episodes were independently counted by two observers using digital counters and stopwatches. The percentage inhibition in cough bouts was calculated for test drug and standard drug in comparison to control group.

Statistical analysis

The results are presented as Mean±standard error of mean (SEM). Data generated during the study was subjected to student's t' test for unpaired data to assess the statistical significance and considered significant at the levels of $p<0.05$.

RESULTS AND DISCUSSION

Both the test drugs showed significant antitussive activity in SO_2 induced cough in mice in comparison to control Groups. SKR1 showed 70% decrease in cough reflex where as SKR 2 showed 56% reduction in cough reflex. When compared with control SKR1 is showing statistically highly significant results when compared with SKR 2.(Table-2)

Table 2: Showing dose and number of cough episodes along with % change in different groups

Groups	Dose	No of Cough Episodes	% Change
Control	-	54.333 ±9.450	-
Standard	0.05ml/10gm	29.83 ± 01.97**	54↓
SKR 1	32.5mg/kg	16.833±2.072***	70↓
SKR 2	32.5mg/kg	24.000±10.396**	56↓

- Statistically insignificant (p> 0.05), * Statistically highly significant (p<0.001)

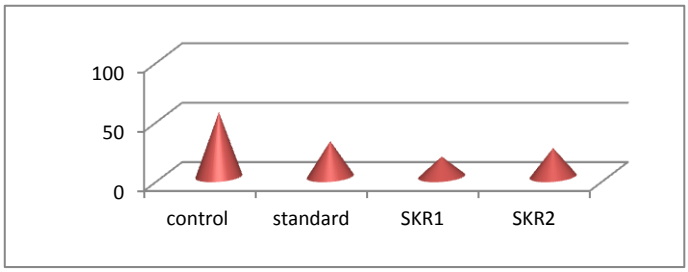
Kajjali is used as a base in maximum formulations of Indian system of medicine. Even though Mercury is a toxic element, the forms of mercury play an important role in converting it to toxic metal. Organic mercury like methyl mercury, ethyl mercury is found to be 5000 times more toxic than inorganic mercury like sulphides of Mercury. Only minimum amount of mercury is absorbed from inorganic mercury [13]. Thus chances of toxicity from *Kajjali* formulations are very negligible or nil since long time these medicines are being used in traditional systems of medicine. The methods of processing the raw material also reduce its toxic effect and make the drug very much suitable for internal use. Present study demonstrated that both the formulations are devoid of any serious toxic effect on acute administration at very high dose of 2000 mg/kg in female rats.

Anti-tussives are cough suppressants. There are two ways to inhibit coughing viz. centrally and peripherally. Centrally acting agents work by inhibiting the cough center in brain, elevating the threshold for coughing. Peripherally acting agents work either by anesthetizing the local nerve endings or acting as demulcents [14].

Both the test drugs showed significant anti-tusstive activity in SO_2 induced cough in mice in comparison to control Groups. The *Shwasakuthara Rasa* containing *Kajjali* showed statistically highly significant (P<0.01) decrease in cough reflexes followed by

Shwasakuthara Rasa without *Kajjali* when compared to control group. The results obtained in both the drugs are better that standard treated group.

SKR 1 contains *Kajjali* which is type of cinnabar used in Chinese medicine more frequently as sedative and hypnotic, thus acting centrally [15]. Traditionally, cinnabar has been used as a tonic to reduce the incidence of palpitations, restlessness and insomnia. *Manahshila* also reported to produce sedative and hypnotic activities in experimental animals [16]. Arsenic preparations most commonly used in diseases of respiratory symptoms. Studies showed that As_2O_3 (Arsenic trioxide) could alleviate the airway inflammation through promoting PE apoptosis and lower PE infiltration. Low dose of As_2O_3 is proved to be effective with relative safety; it also has potential value in treating asthma [17]. Other ingredients like *Piper longum* have shown anti-tussive activity and mast cell stabilizing activity [18]. The drug produced effects through centrally acting [19]. *Piper nigrum* showed significant anti-allergic, anti-asthmatic and anti-inflammatory activity [20]. Study on the effects of ginger and its constituents on airway smooth muscle relaxation and calcium regulation of both human and guinea pig trachea showed significant bronchial relaxant activities. Active component [8]-gingerol protected against methacholine-induced hyperresponsiveness in an *in vivo* murine model [21]. *Z. Officinale* (6-gingerol and 6-shogaol) is also reported for expectorant and anti-tussive activity [22]. Aconite is well known for its anti-inflammatory and analgesic activities. *Tankana*is considered as best expectorant in ayurvedic literature. Thus, there is a synergistic action of individual ingredients in this formulation. Some of the ingredients like *Kajjali, Manahshila* and *Pippali* may be acting centrally and *Manahshila, Maricha, Shunthi, Pippali, Vatsanabha* and *Tankana* may be acting locally in reducing the inflammation and controlling the cough reflex in sulphur dioxide-induced cough reflex in albino mice. (Graph)



Graph 1: Showing the results of Cough reflexes in various groups

Kajjali is a best bioavailability enhancer along with sedative and hypnotic activity. Studies on *Kajjali* also supported its bioavailability enhancing activity [23]. Both the formulations produced significant anti-tussive activity however presence of *Kajjali* in the *Shwasakuthara Rasa* enhance the therapeutic efficacy as anti-tussive drug compared to *Shwasakuthara Rasa* without *Kajjali*. Further, presence of *Kajjali* reduces the overall dose of herbal drugs, constant slow release of herbal drugs and potentiates the therapeutic efficacy.

CONCLUSION

From the present study, it is concluded that *Shwasakuthara Rasa* which is an arseno-mercurial preparation found to be safe at limit dose of 2000mg/kg in acute toxicity study in rats. Both the formations of *Shwasakuthara Rasa* showed significant anti-tussive activity at therapeutic dose level. However, the presence of *Kajjali* in the formulation is safe and further potentiates therapeutic efficacy of the formulation as an anti-tussive agent by its *Yogavahi* (catalytic) activity.

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