Anti-tussive activity of Shwasakuthara Rasa a Herbo-mineral formulation prepared with and without Kajjali (Black Sulphide of Mercury) in SO₂ induced cough in Swiss albino mice

Bhagyalakshmi B R*, Galib R, Mukesh Nariya, Prajapati PK

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 Kaja (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( SO₂).

INTRODUCTION

Mercury is considered as the nucleus of Rasoshadhies (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 Yogavali (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 herb-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 herb-mineral or mineral formulations for antitussive activity; one attempt was made with Sameerapannaga Rasa which is an arsenero-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-tussive 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 Parauda (mercury) and Gandaka (sulphur). To this Kajjali, fine powders of other ingredients like processed Vatsanabha (Aconiumchasmanthum Stupf.), processed Tankaana (Borax), processed Manahshila (Realgar), Maricha (Piperignium Linn.), Pippali (Piperlongum Linn.) and Shunti (Zingiberofficinale Roscoe.) were added one by one and triturated well to get a homogenous mixture. In case of SKR2 fine powders of processed Vatsanabha, Tankaana, 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 1 and SKR 2

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Drug Name</th>
<th>SKR 1</th>
<th>SKR 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kajjali (Black sulphide of Mercury)</td>
<td>2P</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>ShudhaVatsanabha (Processed aconite)</td>
<td>1P</td>
<td>1P</td>
</tr>
<tr>
<td>3</td>
<td>ShudhaTankaana (Processed Borax)</td>
<td>1P</td>
<td>1P</td>
</tr>
<tr>
<td>4</td>
<td>ShudhaManahshila (Processed Realgar)</td>
<td>1P</td>
<td>1P</td>
</tr>
<tr>
<td>5</td>
<td>Maritachooorna (powder of Piperignium Linn.)</td>
<td>9P</td>
<td>9P</td>
</tr>
<tr>
<td>6</td>
<td>PippaliChoorna (powder of Piperlongum Linn.)</td>
<td>1P</td>
<td>1P</td>
</tr>
<tr>
<td>7</td>
<td>ShuntiChoorna (powder of Zingiberofficinale Roscoe.)</td>
<td>1P</td>
<td>1P</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>No of Cough Episodes</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>54.333 ± 49.450</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>0.05ml/10gm</td>
<td>29.83 ± 0.197**</td>
<td>54↓</td>
</tr>
<tr>
<td>SKR 1</td>
<td>32.5mg/kg</td>
<td>16.83±2.072***</td>
<td>70↓</td>
</tr>
<tr>
<td>SKR 2</td>
<td>32.5mg/kg</td>
<td>24.00±10.396***</td>
<td>56↓</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

Both the test drugs showed significant antitussive activity in SO₂ 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).

**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 (NaHSO₃; Nice Chemicals Pvt. Ltd.). Into this bottle, concentrated sulphuric acid was introduced drop by drop. The reaction involved is: 2NaHSO₃+H₂SO₄

\[ 2SO₂+Na₂SO₄+H₂O \]

SO₂ is filled previously in the column of water manometer by opening the three-way cork such that SO₂ 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 SO₂ 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 SO₂ is introduced into the desiccator by this procedure. The mice, after exposure to SO₂ for one minute in the desiccators, were taken out of the desiccator and confined in an upturned 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.
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 As2O3 (Arsenic trioxide) could alleviate the airway inflammation through promoting PE apoptosis and lower PE infiltration. Low dose of As2O3 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. Officinalce (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. Tankanaïs 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 Tankanaïs 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.

REFERENCES


HOW TO CITE THIS ARTICLE