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Sedative-hypnotic Effect of *Ash of Silver* in Mice: A Reverse Pharmacological Study

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ABSTRACT

Ash of silver is used in traditional systems of medicine for various neurological conditions like insomnias, neuralgias, anxiety disorders, and convulsions. The present study was conducted to evaluate the sedative-hypnotic activity of *ash of silver* in comparison to pentobarbitone (standard drug) in albino mice. The mice were divided into four groups as follows: Group 1 (control): Gum acacia [GA; 1% per os (p.o.)], group 2 (standard): Pentobarbitone [50 mg/kg intraperitoneal (i.p.)], group 3 (test): *Ash of silver* (50 mg/kg p.o.), and group 4: *Ash of silver* (50 mg/kg p.o.) given 30 min prior to administration of pentobarbitone (50 mg/kg i.p.). Time of onset, recovery, and total duration of loss of righting reflex were studied. *Ash of silver* (test) produced significant sedation ($P < 0.01$) compared to control (GA 1%), but the effect was significantly less compared to that of standard pentobarbitone at the doses used. Also, significant potentiation ($P < 0.001$) of the sedative-hypnotic effect of pentobarbitone was observed with the test drug.

Key words: *Ash of silver*, Pentobarbitone, Sedation

INTRODUCTION

Traditional systems of medicines have been in use for promotive, preventive, and curative health services since centuries in many parts of the world. Being the oldest traditional system of medicine in India, Ayurveda caters to about 80% of the population in developing countries as per the estimate of World Health Organization (WHO). Despite their wide usage, research in this field is lagging behind with regard to their pharmacologic actions, safety, and efficacy. Ashes or *Bhasmas* used in traditional system of medicine contain heavy metal particles in varying proportions. It is not easy to write off the usage of these preparations just by presuming that heavy metals are toxic. Proper scientific documentation is required to validate the risks and benefits associated with use of such metallic Ayurvedic preparations. There are some specific methods for their detoxification and *Bhasma* preparation, making them suitable for clinical use in therapeutic doses, as claimed by *Rasa Shastra* experts. There is a need to ascertain whether the conventional *Shodhan* (purification) process of Ayurveda is being properly followed or not.[1-3]

Silver is one among the heavy metals which is considered to be a non-essential accumulative trace element with wide distribution in the body, including the central nervous system, but with no known biological and physiological function.[4-6] In Ayurveda, preparations like *Raupya Bhasma* and *Kusta Nukras* have been used to treat many clinical conditions such as pain, inflammation, insomnia, neuralgias, anxiety disorders, convulsions, memory loss, heat stroke, infections, pro-myelocytic leukemias, sexual disorders, etc., for many centuries. Apart from herbs/shrubs, ashes of silver are prescribed. This system also advocates the use of elemental or metallic preparations.[7-9] Metal *Bhasmas* of gold (e.g., gold disodium thiomalate and auranofin) and silver (*Raupya Bhasma,*

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Kusta Nukras, etc.) have been used for the treatment of rheumatoid arthritis, acute pro-myelo cytic leukemias, immunostimulation, and as analgesics in painful inflammatory conditions, and are prescribed with accompaniments such as ginger or cumin water, tulsi extract, lemon extract, etc., that have been shown to protect against unwanted toxicity due to various reasons which include high proportions of trace elements and have synergistic or protective effects due to buffering between various constituents or free radical scavenging property. Oxides of heavy metals are usually not toxic, as claimed by *Rasa Shastra*.[2,10,11]

Since the safety of *ash of silver* has already been established, reverse pharmacological studies are required to confirm the established facts regarding usage, safety, and efficacy in various clinical conditions mentioned above. Since raw silver ore is considered hazardous for health as mentioned in Ayurvedic literature, it needs to be converted into non-harmful form (*ash of silver*) by the process of trituration, pulverization, and repeated calcinations (at 300°C) for 14 times. Reduced form of silver thus obtained acquires spherical nanostructure with a size of 16 nm without any change in the morphology of silver, and is now called as *ash of silver*.[4,7,9] Nanosize of the silver particle is probably responsible for improving the penetration of silver in brain; hence, *ash of silver* had been used in the past for treatment of various neurological conditions, viz. insomnias, anxiety, and pain.[9,10] Being a heavy metal preparation, *ash of silver* bears cumulative potential after prolonged use and in overdoses, as seen in preliminary animal studies. After certain controversial reports of toxicity due to use of metallic/elemental drugs, it has now been made mandatory (WHO guidelines) that Ayurvedic drugs in any form should be tested for their heavy metal content prior to export, so that heavy metals remain within permissible limits.[11-13]

This study was conducted with an aim (a) to explore the sedative-hypnotic effect of *ash of silver*, if any, as claimed in Ayurvedic literature. Further, if *ash of silver* showed sedative-hypnotic effect, the study aimed (b) to observe whether the test drug *ash of silver* was potentiating the sedative-hypnotic effect of pentobarbitone at the doses used in mice when given 30 min prior to the standard drug.

MATERIALS AND METHODS

Swiss albino mice of either sex weighing between 20 and 30 g were screened for the study, after obtaining approval from the Institutional Animal Ethics Committee. Mice were fed on a standard pellet diet and water *ad libitum*, and were housed in polypropylene cages under similar environmental conditions in an animal room that was maintained at 24 ± 1°C and 55 ± 5% humidity with a 12 h light–dark cycle throughout the experiment. In case of oral administration, mice were fasted for 12 h before testing. Plexiglass chamber was used to observe the responses of mice.

Drugs and dosage forms

The test drug *ash of silver* was procured from M/s Baidyanath Ayurved Bhawan Ltd (Jhansi, India). *Ash of silver* [50 mg/kg per os (p.o.)] was suspended in 1% solution of gum acacia. Gum acacia (1% p.o.), procured from Arora Pharmacy (New Delhi, India), was labeled as cont rol and was administered in a volume of 1 ml/100 g. *Ash of silver* and gum acacia were administered orally using infant feeding pipe with a 1 ml syringe attached at the other end. Standard sedative-hypnotic pentobarbitone [50 mg/kg intraperitoneal (i.p.)] was procured from Nembutal Dainippon Pharmaceutical Co. (Osaka, Japan) and was administered as i.p. injection using 1 ml syringe.

Animals and their grouping

Animals (mice) were divided into four groups consisting of six animals in each. Study protocol was as follows:

Group 1: Received vehicle gum acacia (1% p.o.) as control, given in a volume of 1 ml/100 g p.o.

Group 2: Received pentobarbitone (50 mg/kg i.p.) as the standard drug

Group 3: Received the test drug *ash of silver* (50 mg/kg p.o.) suspended in 1% solution of gum acacia

Group 4: Received the test drug *ash of silver* (50 mg/kg p.o.) suspended in 1% solution of gum acacia, following which the standard drug pentobarbitone (50 mg/kg i.p.) was given after 30 min

The responses of all drugs [in terms of time of onset, time of recovery, and total duration of loss of righting reflex (LORR) in mice] were assessed by continuous observation of animals throughout the experiments from the time of administration of drug in the plexiglass chamber, using a stop watch.

Measurement of the duration of pentobarbital-induced LORR

The duration of LORR was measured according to the procedures described by Marley *et al*.[14] Mice were given an i.p. injection of pentobarbitone (50 mg/kg). When the mice became ataxic, they were placed on their backs on a pre-warmed (37°C) pad and the onset, recovery, and total duration of LORR [starting at the time of administration of the test drug (*ash of silver*), the standard drug pentobarbitone (50 mg/kg), and the test drug (*ash of silver*) followed 30 min later by the standard drug pentobarbitone (50 mg/kg)] were noted until they regained their righting reflexes. Mice were presumed to have regained the righting response when they could right themselves three times within 30 sec.

RESULTS

Findings of the present study are depicted in Table 1.

All values were expressed as Mean ± SEM and analyzed using analysis of variance (ANOVA) followed by Dunnett's "*t*" test. $P < 0.05$ was considered significant.

DISCUSSION

In the present study, we tried to explore the pharmacological effect of *ash of silver* as a sedative-hypnotic and its secondary effect to potentiate the sedative-hypnotic effect of pentobarbitone. Such studies help to fast track drug discovery and development when carried out in selected animal models through screening. Thus, Ayurvedic knowledge and experimental database are able to provide new functional leads, thereby reducing the toxicity of drugs and saving time and money.[13]

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