Indian Journal of Pharmaceutical Education and Research | Association of Pharmaceutical Teachers of India

Evaluation of Effect of Shodhana Treatment on Pharmacological Activities of Aconite

P.K. Sarkar[1]*, P.K. Prajapati[2], V.J. Shukla[2] and B. Ravishankar[2]

[1]Department of Rasashastra, J. B. Roy State Ayurvedic Medical College & Hospital, 170-172, Raja Dinendra Street, Kolkata (India)

[2]Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat (India)

ABSTRACT

Submitted: 18/02/2011 Revised: 24/04/2011 Accepted: 18/10/2011

The lethal plant origin cardiac and neuro-muscular poison 'aconite' is used as a therapeutic agent for the treatment of fever, arthritis, in Ayurvedic system of medicine after Shodhana treatment. The present study was designed to evaluate likely impact of Shodhana treatment on cardiac activity, antipyretic activity and neuro-muscular activity of aconite. Pharmacological activities of raw aconite (RV), treated aconite in cow's urine (SM) and treated aconite in cow's milk (SD) were evaluated and compared. Cardiac activity study was done on Charles Foster albino rats by taking ECG. Antipyretic study was carried out on yeast induced pyrexia in Wistar rats. In vitro neuro-muscular activity study was done on leech dorsal muscle by recording contractions. Increase in the heart rate, prolongation of the duration of the QRS complex and prolongation of the repolarizaion period between two QRS complexes were found in RV treated group. No such changes were observed in SM and SD treated groups. A significant ($P<0.05$) body temperature lowering effect was found in RV treated group, the activity was found less in SM and SD treated groups. Muscle relaxant activity of RV and myo-contraction activity of SM and SD was noticed. Shodhana treatment removes cardiac and neuro-muscular toxic effect from raw aconite without affecting antipyretic activity in a drastic manner.

Keywords: Aconite, antipyretic, cardiac, neuro-muscular.

INTRODUCTION

Aconite is considered as one of the most lethal plant origin poisons. It produces toxicity mostly in the cardio-vascular system and neuro-muscular system. But the Ayurvedic science of medicine looks upon it as a therapeutic entity. It is used in therapeutics after a special processing called as 'Shodhana'. The treated aconite is used for treatment of fever, arthritis, etc[1].

Aconite is reported to have depressant action on mammalian heart. It stimulates the vagus centre and slows the heart rate. It induces cardiac arrhythmias, ventricular fibrillation and ventricular tachycardia[2]. Some study reported cardiac stimulant activity of processed aconite[3]. Aconite has a characteristic action on all the sensory nerves, at first the nerve endings are stimulated with the characteristic prickling and tingling sensation. After a short time the action is reversed and the nerve endings are paralyzed and symptoms like numbness occurs[4]. Aconites are found to have potent antipyretic effect; the onset of action is quick, but the duration of action is shorter, which is comparable to the action of paracetamol[5].

The present study was carried out to observe the cardiac activity, neuro-muscular activity and anti-pyretic activity of raw aconite (RV), processed aconite in cow's urine (SM) and processed aconite in cow's milk (SD) with emphasis to prove the need and importance of the Shodhana procedure done for the poisonous plant drugs in Ayurvedic system of medicine. The study confirms to the guidelines laid by the Institutional Animals Ethics Committee (IAEC-06-08/02).

***Address for Correspondence:**
P.K. Sarkar, H/144, Netaji Colony, Noapara, Kolkata- 700090, West Bengal
E-mail: prasantaksarkar@yahoo.com, prasantsarkar@indiatimes.com

MATERIALS AND METHODS

Cardiac activity study:

***Animals*:**

Charles Foster strain albino rats of either sex weighing between 180 g to 250 g were used for experiments. They were obtained from the Animal House attached to the pharmacology laboratory. They were housed in breeding cages at an ambient temperature with a natural day and night cycles. The animals had free access to Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water.

***Experimental design*:**

The cardiac activity was evaluated by taking ECG after administration of test drugs. The ECG was taken on anesthetized rats. The rats were anesthetized by using diethyl ether. ECG was recorded by using a portable electrocardiogram machine. Only the four standard leads were attached to the four extremities of the animals, the chest leads were not used. The paper speed of the ECG machine was set to 50/s. The parameters like heart rate, duration of QRS complex and R-R interval was counted from lead II of the ECG[6].

RV, SM and SD were made into fine powder form, and drug suspension was prepared by adding 1 ml of 5% gum acacia solution in 100 ml of distilled water.

***Study protocol*:**

Total 24 Charles Foster rats of either sex weighing between 180 g to 250 g were taken and divided randomly into 6 groups, each containing 6 animals, 3 male and 3 female. The treatment schedule was as follows, group I comprised of vehicle (tap water) treated control animals, group II animals were treated with 6.75 mg/kg, suspension of RV, group III animals received 6.75 mg/kg, suspension of SM and animals of group IV were treated with 6.75 mg/kg, suspension of SD. The treatment schedule was continued for 30 days and ECG was taken after 1 h of drug administration on 1st, 15th and on 30th day.

Antipyretic study:

***Animals*:**

Wistar strain albino rats of either sex weighing between 180 g to 250 g were used for experiment. They were obtained from the Animal House attached to the pharmacology laboratory. They were housed in breeding cages at an ambient temperature with a natural day and night cycles. The animals had free access to Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water.

***Experimental design*:**

Pyrexia was induced by s.c. injection of 1 ml/100 g of 12.5 % yeast suspension in normal saline solution. RV, SM and SD were made into fine powder form, and drug suspension was prepared by adding 0.5 ml of 5% gum acacia solution in 20 ml of distilled water. Paracetamol tablets were made into fine powder and solution was prepared by adding distilled water. The drugs were administered orally 1 h before yeast administration. The rectal temperatures were recorded by using digital telethermometer after 3 h, 6 h, 9 h and 24 h of yeast administration[7].

***Study protocol*:**

Total 24 Wistar rats of either sex weighing between 180 g to 250 g were taken and divided randomly into 4 groups, each containing 6 animals, 3 male and 3 female. All the rats were injected s.c. by 1 ml/100 g of yeast suspension. The treatment schedule was as follows, group I comprised of only yeast treated vehicle control animals; group II animals were treated with 40.0 mg/kg, solution of paracetamol; the animals of group III were treated with 1.35 mg/kg, suspension of RV, group IV animals received 1.35 mg/kg, suspension of SM and group V animals were treated with 1.35 mg/kg, suspension of SD.

***In vitro* neuro-muscular activity study:**

***Experimental design*:**

In vitro neuro-muscular activity study was done on isolated dorsal muscle of leech (*Hirudo medicinalis*). A leech was pinned on its back through the mouth and tail-suckers on to a cork board. A cut was made with scissors along the two pale lateral lines, from the mouth to the tail, and the internal organs were removed. The dorsal muscle was then pinned out at either side and divided longitudinally, and threads were attached at the top and bottom of each piece. The muscle was suspended at room temperature in Frog-Ringer solution, It was prepared by adding NaCl (6.5 g), KCl (0.14 g), $CaCl_2$ (0.12 g), $NaHCO_3$ (0.2 g) and glucose (2 g) to 1 L of distilled water, through which oxygen was blown. One thread was tied to the pin in the organ bath and the other to the lever. The load on the lever was 2.5 g and the magnification was about 10-fold. The capacity of the organ bath was 40 ml. The effect was measured by the mark on the smoked paper attached to the drum of a kymograph made by a simple sideways-writing lever, and a permanent record of the experiment was obtained by varnishing the paper[8].

RV, SM and SD were made into fine powder form, and drug suspension was prepared by adding 1 ml of 5% gum acacia solution in 100 ml of distilled water. Suspensions of RV, SM and SD were prepared in 10 mg/ml concentration.

***Study protocol*:**

Responses of RV (25 μg), SM (25 μg) and SD (25 μg) were recorded. The drugs were allowed to be in contact with the leech dorsal muscle for 8 m and then responses of muscle were recorded for 2 m. The muscle was allowed to relax for 15 m between two responses.

Statistical analysis:

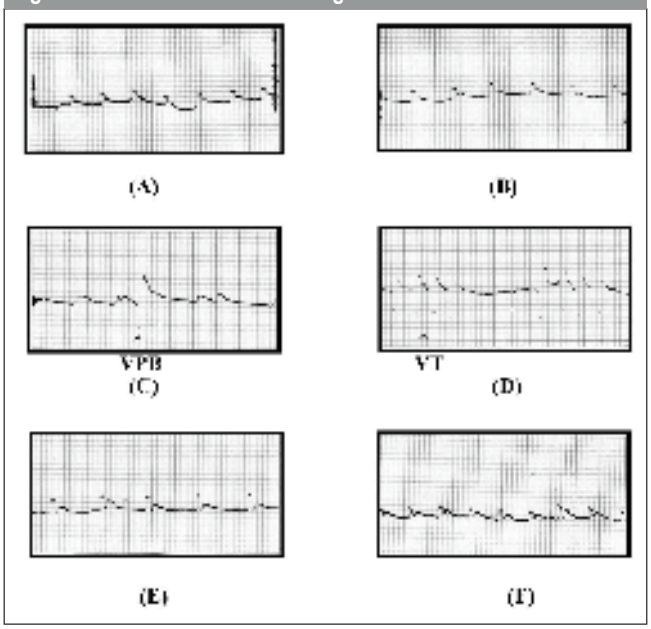
All the values were expressed as mean ± SEM (standard error of mean). The data were analyzed by paired and unpaired 't' test and by one way ANOVA. A level of $P<0.05$ and $P<0.01$ was considered as statistically significant and highly significant respectively.

RESULTS

Cardiac activity study:

Effect of administration of all the test drugs was evaluated on 3 parameters of ECG i.e. heart rate, QRS complex and repolarization time. Among the 3 drugs studied along with vehicle control group, significant changes in ECG were found only in RV treated group. Significant decrease in heart rate after 15 and 30 days of drugs administration, significant increase in QRS complex after initial and 30 days of treatment and highly significant and a huge increase in repolarization time after 15 days and 30 days of drug administration respectively were the significant changes observed in the ECG of RV administered group. Ventricular premature beat (VPB) and ventricular tachyarrhythmia (VT) were diagnosed in the clinical interpretation of the ECG of the RV treated rats after 15 and 30 days of treatment presented in figure. 1.

Fig. 1: ECG of control and test drugs treated rats.



It was observed that after 30 days of treatment by SM and SD, the heart rates were higher by 27.78 % and 32.13 % respectively in comparison to RV treated group. The prolongation in the QRS duration of SM and SD treated groups was much less in comparison to the increase observed in RV treated group. The prolongation in time between two repolarisation phases of heart in SM and SD treated groups after 15 days of treatment was only marginal in comparison to control group and was statistically non-significant. The difference between the RV group and SM and SD groups was found to be statistically significant (Table 1).

Antipyretic study:

The data pertaining to the effect of test drugs on body temperature in yeast induced pyrexia in rats have been summarized in Table 2. Injection of yeast induces significant elevation in rectal temperature. All the test drugs exhibited body temperature lowering activity. After 3 h of drug administration all the test drugs and paracetamol showed significant to highly significant decrease in body temperature. None of the test drugs even paracetamol also could decrease the body temperature significantly after 6 h of drug administration. After 9 h of drug administration only paracetamol administered group showed significant decrease of body temperature. After 24 h of drug treatment RV, SD and paracetamol exhibited significant body temperature lowering effect.

***In vitro* neuro-muscular activity study:**

In vitro neuro-muscular activity study of RV, ST and SD suspensions in leech dorsal muscle reveals that application of 0.1 ml of RV suspension (25 µg/ml of bath fluid) caused

Table 1: Effect of raw and treated aconite on heart rate, QRS complex and repolarization time in rats

Group	Dose (mg/kg)	Heart rate (/min)			QRS complex (sec)			Repolarization time (sec)		
		1st day	15th day	30th day	1st day	15th day	30th day	1st day	15th day	30th day
Control	-	403.33 ± 29.97	413.83 ± 20.34	406.83 ± 23.90	0.083 ± 0.003	0.103 ± 0.008	0.093 ± 0.007	0.150 ± 0.013	0.145 ± 0.007	0.148 ± 0.009
RV	6.75	380.50 ± 19.39	317.50 ± 10.40$^{**\#\#}$	286.83 ± 39.30$^{**\#\#}$	0.100 ± 0.007$^{**\#}$	0.097 ± 0.008	0.147 ± 0.020$^{**\#\#}$	0.158 ± 0.010	0.190 ± 0.007$^{**\#}$	0.237 ± 0.041$^{\#\#}$
SM	6.75	380.83 ± 31.89	395.00 ± 16.44	366.50 ± 22.02	0.087 ± 0.004	0.093 ± 0.007	0.103 ± 0.008	0.160 ± 0.013	0.152 ± 0.007^{**a}	0.165 ± 0.011
SD	6.75	399.83 ± 26.64	390.83 ± 26.18	379.00 ± 17.57	0.090 ± 0.007	0.097 ± 0.008	0.097 ± 0.008^{**a}	0.152 ± 0.010	0.155 ± 0.010^{**a}	0.158 ± 0.008

Values are expressed as Mean ± SEM (standard error of mean). Number of animals (n = 6). *= P<0.05; **= P<0.01 by the student's 't' test; $^{\#}P \leqslant 0.05$; $^{\#\#}P \leqslant 0.01$ by the multiple 't' test; ^{**a} = when compared to RV treated group.

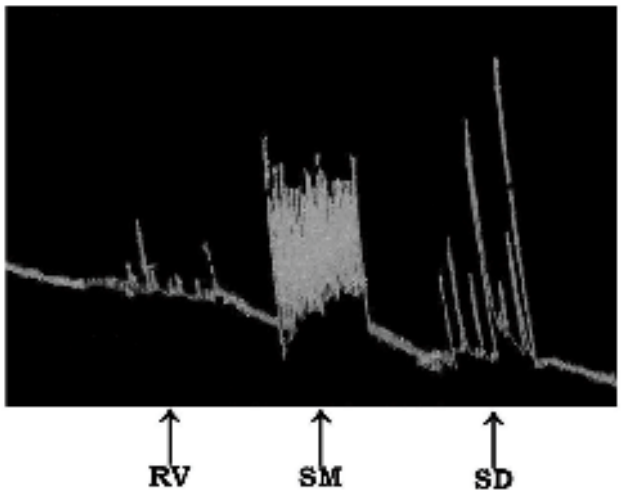
Table 2: Effect of test drugs on yeast induced pyrexia in rats

Group	Dose (mg/kg)	Temperature (^{o}F)				
		Initial	3 h	6 h	9 h	24 h
Vehicle	-	37.80 ± 0.32	39.53 ± 0.39*z	39.64 ± 0.33*z	39.95 ± 0.33*z	40.26 ± 0.53*z
Paracetamol	40.0	37.43 ± 0.15	37.50 ± 0.27 **	38.85 ± 0.38	38.33 ± 0.34 **	38.13 ± 0.31 **
RV	1.35	37.53 ± 0.16	37.92 ± 0.19 **	39.43 ± 0.22	39.50 ± 0.24	38.62 ± 0.22 *
SM	1.35	37.82 ± 0.16	38.42 ± 0.12 *	39.55 ± 0.11	39.70 ± 0.15	39.10 ± 0.15
SD	1.35	37.70 ± 0.18	38.40 ± 0.12 *	39.37 ± 0.11	39.43 ± 0.15	38.53 ± 0.09 **

Values are expressed as Mean ± SEM (standard error of mean). Number of animals (n = 6). *= P<0.05; **= P<0.01; *z: P <0.05, when compared to initial values.

relaxation of the dorsal muscle, whereas contraction responses were seen in dorsal muscle of leech after application of 0.1 ml of SM and SD suspension (25 µg /ml of bath fluid). The contraction responses of SM were found to be better than contraction responses of SD (Figure. 2).

Fig. 2: Kymograph of test drugs treated contractions of leech dorsal muscle.



DISCUSSION

In the cardio-protective study the effects of RV were compared to the effect produced by SM and SD to get an idea about the likely effect of the Shodhana treatment in removing the cardio-toxic principles from RV. Careful analysis of the results indicates that as the duration of administration of RV increased, the cardio-toxic activity also increased. The major effect observed were increase in the heart rate, prolongation of the duration of the QRS complex and prolongation of the repolarizaion period between two QRS complexes. These three toxic changes were much less in SM and SD treated groups. This clearly shows that the cardio-toxicity producing principle observed in RV is removed by Shodhana process.

The aconite is found to have a depressant action on mammalian heart. It stimulates the vagus centre and slows the heart rate[9]. This may be reason behind decreased heart rate in RV treated animals. The QRS complex is produced by ventricular depolarization. The increase in QRS complex occurs due to intra-ventricular conduction defect and in bundle branch block. The others changes in bundle branch block like axis deviation, altered RSR[1] pattern were not found in ECG of RV treated animals. During repolarisation the return of myocardial fibres from stimulation during depolarization to their original resting state occurs. Increase in repolarisation time indicates elevation in resting phase of the myocardial fibres and occur due to conduction defect. From the observed changes in ECG of RV treated group, it may be inferred that the alterations occur due to ventricular conduction defect.

The ventricular premature beat and ventricular tachyarrhythmia diagnosed in the clinical interpretation of the ECG of the RV treated rats after 15 and 30 days of treatment may occur due to disorders of impulse propagation. The alkaloids from *Aconitum* sp. induce an increase in synaptosomal sodium [Na^{+}] and calcium [Ca^{2+}] ions, cause conduction defects and provoke tachyarrhythmia[10].

All these parameters were not changed to significant level in the SM and SD treated groups, in comparison to vehicle control group. The ST segment was raised in SD treated group, even in control group also the ST segment was raised, and thus any pathological intervention could be excluded.

Aconites are found to have potent antipyretic effect; the aconitine group of alkaloids has the body temperature lowering effect[5]. The result of the antipyretic study also supports this activity of RV. A significant body temperature lowering effect was found in RV treated group; even the antipyretic activity of RV was comparable to that of paracetamol. The body temperature lowering activity was found to be less in SM and SD treated group in comparison to RV treated group. In this respect SD was found to be a better antipyretic agent than SM.

After Shodhana treatment of aconite the total alkaloids

content was reduced to 80 percent. Aconitine content of SD was found to reduce by 70 percent and no trace of aconitine was found in SM[11]. These facts may be considered as the cause behind low antipyretic activity exhibited by SM and SD, and better result of SD as antipyretic agent than SM. The SD may be recommended to use as anti-pyretic instead of RV, because this has clear advantages in that by using SD, potential toxic effects of RV can be avoided.

Raw aconite causes loss of muscle tone, balance and coordination in mice[12]. In this study, muscle relaxant activity of RV in leech dorsal muscle was observed, whereas contraction activity was noticed by treatment of SM and SD. It reveals that myo-relaxant activity of RV was reversed after Shodhana treatment and myo-contraction activity occurs.

CONCLUSION

This study provides clear evidence for the efficacy of the Shodhana process in removing toxic principles from raw aconite, which are mainly cardiac and neuro-muscular toxic aconitine related alkaloids, and it is also proved that Shodhana process though leads to some loss of antipyretic activity will not remove the benefit in a drastic manner.

ACKNOWLEDGEMENT

The authors thank Prof. M. S. Baghel, Director, I. P. G. T. & R. A., Gujarat Ayurved University for extending all necessary facilities and Dr. Ashok B. K. for support to carry out the experiments.

REFERENCES

1. Mishra GS, Madhav Upadhyay's Ayurved Prakash. New Delhi, Chawkhamba Bharati Academy. 1994, 486-98.
2. Tai YT, But PP, Young K, Lau CP, Cardiotoxicity after Accidental Herb-Induced Aconite Poisoning. Lancet 1992;340:1254-6.
3. Handa KL, Chopra IC, Kohli JD, Singh K, Mitigation of Aconite, A preliminary note, Indian J Med Res 1951,39:89-98.
4. Dzhakhangirov FN, Bessonova IA, Alkaloids of *Aconitum coreanum* X. Curare like Activity Structure Relationship. Chem Natural Compounds 2002;38:74-7.
5. Singh LB, Singh RS, Bose R, Sen SP, Effect of Shodhana of aconite on its pharmacological action. Indian J Pharmacol 1981;13:93-7.
6. Apte K, Hede S, Cardiovascular Effects of Cetyl Trimethyl Ammonium Bromide- Protected Gold Nanoparticles. Indian J Pharmacol 2007;39:210-3.
7. Pal SC, Nandy A, Antiinflammatory, Analgesic and Antipyretic Activity of Achras sapota Linn. Leaf Extracts and Its Isolated Compounds. Indian Drugs 1999;32:106-13.
8. Sheth UK, Dadkar NK, Kamat UG, Selected Topics in Experimental Pharmacolody. Bombay, The Kothari Book Depot. 1972. 52.
9. Fu M, Wu M, Qiao Y, Wang Z, Toxicological Mechanisms of Aconitum Alkaloids. Pharmazie 2006;61:735-41.
10. Friese J, Gleitz J, Guster UT, Heubach JF, Matthiesen Tb, Wilffertc B, Selve N, *Aconitum* sp. alkaloids: the modulation of voltage-dependent Na^+ channels, toxicity and antinociceptive properties. European J Pharmacol 1997;337:165-74.
11. Sarkar PK, Prajapati PK, Ravishankar B, Evaluation of Shodhana process and antidotal studies on Vatsanabha [dissertation]. Jamnagar, Gujarat Ayurved University; 2008.
12. Thorat S, Dahanukar S, Can We Dispense with Ayurvedic Samskaras? J Postgrad Med 1991;37:157-9.

****