

Research Article

EFFECTS OF AVURVEDIC SHODHANA (PROCESSING) ON DRIED TUBEROUS ACONITE (*Aconitum napellus* Linn.) ROOT

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ABSTRACT

Aconite (*Aconitum napellus* Linn.) commonly known as atis is a poisonous plant used extensively as antihypertensive, antipyretic, analgesic and antirheumatic. Ayurveda recommended the administration of aconite roots only after purification, i.e., boiling roots in cow's urine (Gomutra). In the present study an attempt was made to compare the pro-arrhythmic and antihypertensive effects of powdered aconite root purified by shodhana process with that of unpurified form of aconite roots in order to provide scientific support of the claim in ayurvedic texts that purification of aconite root by shodhana process retains its antihypertensive activity and is devoid of pro-arrhythmic activity. Aconite root treatment in both forms (purified and unpurified) caused significant reduction in BP when compared with diseased control group ($P < 0.05$). The unpurified aconite root group showed significant increase in heart rate, increase in QRS complex time and increase in QT interval, however these parameters were statistically insignificant in purified aconite root treated group. The PRA, SC and BUN levels was significantly decreased in aconite root treatment groups. The probable mechanism of antihypertensive activity of aconite root can be attributed to decrease in plasma renin activity, decrease in oxidative stress and increase in NO levels.

Key words: Aconite, shodhana process, antihypertensive, proarrhythmic

INTRODUCTION

In Ayurveda, the ancient Indian system of medicine mainly deals with herbal and herbo- mineral formulations to cure the pathological symptoms as well as to maintain good health. Some of the most important herbal drugs are toxic in nature and hence Ayurveda has classified such drugs in the *Visha-Upavisha Varga* (poisonous and semi poisonous drugs) and mentions the *Shodhana samskara* (process of detoxification) for the same to reduce their toxicity and potentiate their medicinal properties (Medhava and Mishra, 1999; Sharma, 2001). According to ayurvedic texts shodhan vidhi is an important process which enhances the biological activity of a compound and reduces the toxicity at the same time. Before incorporating into formulations, guggul is processed using Shodhan vidhi involving different shodhan dravyas like gulvel, gomutra, triphala, dashmul (Kamble *et al.*, 2008).

Raw aconite is an extremely lethal poison. Aconite's action is primarily cardiotoxic because of its effect on the inward sodium channels. It prolongs cardiac repolarization, and as a result, various arrhythmias-primarily ventricular-can occur. It is also neurotoxic (Rastogi *et al.*, 2007; Singh *et al.*, 1986; Chan, 2009). However, the science of ayurveda looks upon it as a therapeutic entity. The ayurvedic science is full of pharmaceutical processing also called as 'Samaskara'. shodhana treatment is also one type of samaskara. Raw aconite is always subjected to shodhana before being utilized in the ayurvedic formulations, and after shodhana treatment it is prescribed to combat various illnesses like fever, rheumatoid arthritis, sciatica, hypertension etc (Toshiaki *et al.*, 2009; Rastogi, 2011). Some of the ayurvedic formulations containing aconite roots are Mahavisagarbha Taila, Rodhrasava, Lakasminarayana Rasa and Sudarsana Churna (Rastogi, 2011).

In the present study, aconite was purified by cow's urine (shodhana) as described in ayurvedic text. In order to claim the detoxification effect of shodhana process on aconite, the aconite roots was studied for its pro-arrhythmic and antihypertensive activity in both forms; i.e., raw aconite and shodhana treated aconite roots (Rastogi, 2011; Singh *et al.*, 1981; Singh *et al.*, 1981).

METHODOLOGY

Materials

The dried roots of Aconite (*Aconitum napellus* Linn.) was procured from Sanjivani Aaushdhalaya, Bhavnagar, Gujarat, India and was authenticated by Dr. P.S. Nagar, Head, Department of Botany, M.S. University of Baroda, Vadodara, Gujarat, India. A voucher specimen of the drug was kept for future reference (bot/bio/aut/09212/06). Roots of Aconite (*Aconitum napellus* Linn.) was purified by shodhana process using cow's urine collected from local cow shed. Aconite roots (250g) were cut into small pieces and was placed into Dolayantra (an earthen pot of 10L capacity), which was filled with cow's urine. Dolayantra was placed on fire and heated for 3h daily for continuous three days. After three days the aconite roots were collected and washed thoroughly three times with cold water and dried under sun light. After drying roots were powdered (Thorat and Dahanukar, 1999; Sarkar and Prajapati, 2011). Olive oil suspensions of powdered aconite root of both purified and unpurified was prepared freshly before the administration of drug.

Instruments: UV-Vis spectrophotometer (Specord, Analytic Jena, Germany), Homogeniser (Remi), High Speed Cooling Microcentrifuge (Remi C-24) and Student's Physiograph (Biodevices) alongwith strain gauge coupler, pressure transducer, ECG coupler, ECG leads.

Chemicals: AR grade ether, olive oil, thiobarbituric acid, Tris buffer, trichloroacetic acid, griess reagent, ethylenediamine tetraacetic acid and sodium nitrite were obtained from Qualikems Fine Chem. Pvt Ltd., Vadodara.

Animals

Adult wistar rats of either sex weighing between 250-300g were procured from animal

house of Babaria Institute of Pharmacy, Vadodara. Approval of animal experimental protocol was taken by Institutional Animal Ethics Committee of Babaria Institute of Pharmacy, Vadodara (M.Ph.Sem-IV/11-12/04). Rats were housed in polypropylene cages with rice husk as the bedding material maintained under standardized condition (12h light/dark cycle, $24\pm 1^{\circ}\text{C}$, 35- 60% humidity) and provided with free access to commercial pellet diet (Mfg. by Nav Maharashtra Chakan Oil Mills Pvt. Ltd., Pune) and water *ad libitum*. Rats were randomly assigned to four groups with six animals in each group, viz., Normal Control (Olive oil 1ml, p.o.); Diseased control; Unpurified aconite root 50mg/kg, p.o. and Purified aconite root 50mg/kg, p.o. The animals were treated with drug for 6days.

Measurement of blood pressure and pro-arrhythmic activity of powdered aconite roots against two kidney one clip renovascular hypertension model in rats (Goldbatt *et al.*, 1934; Kotrly *et al.*, 1984).

Each day ECG in lead II were recorded on student physiograph (Biodevices). From ECG recording, the heart rate (beats/min), QRS complex time (in secs) and QT interval (in secs) was calculated. On 7th day, animals were anesthetized with ketamine (50mg/kg, i.p.) alongwith diazepam (5mg/kg). A PVC-coated Dieffenbach clip was placed onto the left hilum of the kidney and fixed to the back muscles. A small cut was made on the left side of the abdomen. Through this incision left kidney is located and the renal artery was occluded for 3½ h. Carotid artery was cannulated with 50 PE tube. The trachea was cannulated to facilitate spontaneous respiration. To measure systolic and diastolic blood pressure, the cannula in the carotid artery was connected to a pressure transducer (student's physiograph). After recording stable reduced blood pressure values, the renal arterial clip was removed. This leads to a rise in blood pressure as a consequence of elevated plasma renin level. Blood pressure was recorded & monitored continuously. Treatment groups were compared with disease control group. The purified aconite root treatments group was compared with unpurified aconite root treated groups.

Table I. Effect of aconite on blood pressure

Treatment	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Normal control	123.0 ± 3.7	66.7 ± 2.5
Disease control (Sham operated)	156.2 ± 4.2 [§]	97.7 ± 2.0 [§]
Aconite unpurified (50mg/kg, p.o)	140.0 ± 2.6 [@]	80.2 ± 1.5 [@]
Aconite purified (50mg/kg, p.o)	137.5 ± 1.8 [@]	76.0 ± 1.6 [@]

Values are Mean ± S.E.M. (n=6).

[§] P < 0.05 when compared to normal control group

[@] P < 0.05 when compared to diseased control group

ANOVA one way followed by Bonferoni's post test

Table II. Effect of aconite on ECG

Treatment	Heart rate (beats/min)	QRS complex (Sec)	Q-T interval (Sec)
Normal control	359.8 ± 18.9	0.023 ± 0.001	0.068 ± 0.020
Aconite unpurified (50mg/kg, p.o)	480.0 ± 13.8 [@]	0.042 ± 0.007 [@]	0.084 ± 0.001 [@]
Aconite purified (50mg/kg, p.o)	375.7 ± 17.9 [*]	0.029 ± 0.003 [*]	0.067 ± 0.003 [*]

Values are Mean ± S.E.M. (n= 6).

[@] P < 0.05 when compared to normal control group

^{*} P < 0.05 when compared to unpurified aconite treated group.

ANOVA one way followed by Bonferoni's post test

At the end of experiment the blood sample of 2 ml was collected by retro-orbital puncture and allowed to clot for 10min at room temperature. Clots were centrifuged at 2500rpm for 10min to separate the serum. Serum creatinine (Kaplan and Pesce *et al.*, 1996), Blood Urea Nitrogen (BUN) (Godkar and Godkar, 2009) and Plasma renin activity (PRA) (Wang *et al.*, 1993) were measured by commercial available kits (Transasia Bio-Medical Ltd). Animals were sacrificed and the left kidney was quickly removed, weighed & perfused immediately with ice cold hypertonic saline solution, weighed and homogenized in chilled Tris- buffer (10mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 rpm at 0°C for 20min using Remi C-24 high speed cooling microcentrifuge. The clear supernatant was used for the assay of Nitrite (NO) (Lepoivre and Chenais, 1990), Malondialdehyde (MDA) (Ohkawa *et al.*, 1979), Catalase (CAT) (Aebi, 1984), Reduced Glutathione (GSH) (Moran *et al.*, 1979) and Superoxide Dismutase (SOD) (Nandi and Chatterjee, 1988).

Statistical Analysis of Results

All values were expressed as Mean ± SEM. Statistical significance was tested between more than two groups using one-way ANOVA followed by Bonferoni's post test by using a computer-based fitting program (Prism, Graph pad 5). Differences were considered to be statistically significant when P < 0.05.

RESULTS AND DISCUSSION

Effect of aconite on blood pressure

Systolic and diastolic blood pressure were found to be statistically significant (P<0.05) increased in diseased control group as compared to normal control group that indicated hypertension in diseased control rats. Aconite root treatment (purified and unpurified) caused significant reduction in systolic & diastolic blood pressure when compared with diseased control group (P<0.05). However, when unpurified aconite root treated group was compared with that of unpurified aconite treatment group; no statistically significant change in systolic and diastolic blood pressure was observed (Table I).

Table III. Effect of aconite root treatment on Plasma Rennin Activity (PRA), serum creatinine, Blood Urea Nitrogen(BUN) and Nitric Oxide(NO).

Treatment	Plasma Renin activity (ng/mL/hr)	Serum creatinine mg/dL	BUN mg/dL
Normal Control	5.50 ± 0.67	1.13 ± 0.14	31.00 ± 3.12
Disease Control	14.33 ± 1.23 [§]	2.70 ± 0.24 [§]	52.17 ± 3.40 [§]
Aconite unpurified (50mg/kg, p.o)	9.83 ± 1.26 [@]	1.73 ± 0.15 [@]	38.50 ± 1.73 [@]
Aconite purified (50mg/kg, p.o)	9.33 ± 0.88 [@]	1.63 ± 0.13 [@]	38.33 ± 1.17 [@]

Values are Mean ± S.E.M. (n=6).

[§] P < 0.05 when compared to normal control group

[@] P < 0.05 when compared to diseased control group

ANOVA one way followed by Bonferoni's post test

This particular observation suggests that the purification of aconite by shodhana process retains the antihypertensive activity.

Effect of aconite on ECG

When Aconite drug treatment group (both purified and unpurified) were compared with normal control group, statistical significant (P<0.05) increase in heart rate, increase in QRS complex time and increase in QT interval was observed only in unpurified aconite root treated group. The changes in heart rate, QRS and QT time in purified aconite root treated group was not found to be significant (Table II). This indicates that shodhana purified aconite root is devoid of the pro-arrhythmic activity and hence a strong evidence of the ayurvedic claim that shodhana process of aconite using cow's urine diminishes the pro-arrhythmic effect.

Unpurified Aconite contains aconitine which is highly toxic to the heart. Aconitine blocks HERG and K_{v1.5} potassium channels in the open state. Blockage of potassium channels, particular the HERG channel may be one of the important mechanisms of how aconitine induces arrhythmias. Aconite is purified by shodhana using cow's urine. After sodhana process the aconitine converts into aconine, 16-epidesbenzoyl pyroaconine, and the major hydrolysis product is benzoylaconine. Thus the Aconitine concentration is reduced after purification (Li and Tu, 2010).

Effect of aconite on plasma renin activity (PRA), serum creatinine, blood urea nitrogen

In order to elucidate the probable mechanism of the antihypertensive activity of the aconite root, various markers for the antihypertensive activity is also determined. The plasma renin activity, serum creatinine and blood urea nitrogen was significantly decreased in aconite root treatment groups when compared with diseased control group (P<0.05). However, no significant change was observed between aconite treatment groups for these serum parameters (Table III). The renin-angiotensin system (RAS) plays a dominant role in the regulation of the blood pressure. Angiotensin converting enzyme (ACE) is the most important regulatory site of RAS. The importance of ACE inhibitors in the chronic treatment of various cardiovascular diseases such as hypertension, congestive heart failure, myocardial infarction, diabetic nephropathy, or renal dysfunction is now well established.

The present study showed an anti-hypertensive effect of Unpurified and Purified sample of Aconite in two kidney one clip (2K1C) renovascular hypertension. The 2K1C model is characterized by an increase in blood pressure immediately after clipping, which parallels the release of active renin concentration. The plasma and renal renin levels were found to increase in both the early and chronic phase of 2K1C hypertensive rats.

Table IV. Effect of aconite on oxidative stress markers in kidney tissue.

Treatment	CAT μM of H ₂ O ₂ consumed/ g of tissue	GSH μg/g of tissue	SOD Units/g of tissue	MDA nM/g of tissue	NO μM/g of tissue
Normal Control	321.7 ± 6.2	148.2 ± 10.1	104.2 ± 6.8	73.00 ± 3.9	8.95 ± 1.29
Disease Control	199.2 ± 11.6 [§]	79.29 ± 7.4 [§]	45.83 ± 6.5 [§]	125.8 ± 5.8 [§]	1.95 ± 0.27 [§]
Aconite unpurified (50mg/kg, p.o)	265.8 ± 4.7 [@]	118.3 ± 5.4 [@]	76.33 ± 5.7 [@]	96.67 ± 5.7 [@]	4.05 ± 0.35 [@]
Aconite purified (50mg/kg, p.o)	270.8 ± 4.7 [@]	124.3 ± 5.1 [@]	80.83 ± 4.7 [@]	93.33 ± 4.4 [@]	5.17 ± 0.30 ^{@*}

Values are Mean ± S.E.M. (n=6).

[§] P < 0.05 when compared to normal control group

[@] P < 0.05 when compared to diseased control group

* P < 0.05 when compared to unpurified aconite treated group.

CAT: catalase; **GSH**: glutathione; **SOD**: superoxide dismutase; **NO**: nitric oxide

ANOVA one way followed by Bonferoni's post test

The ACE is the most important regulatory site of RAS. The ACE plays an important role in the regulation of blood pressure and diuresis. It catalyzes the hydrolysis of Angiotensin-I to Angiotensin-II, which is a powerful vasoconstrictor. Angiotensin-II also increases the synthesis and release of aldosterone, which cause sodium and water retention. Aconite powder blocked the increase of blood pressure in 2K1C hypertensive rats. These results suggest that Aconite powder has an antihypertensive effect via the inhibition of ACE, an essential enzyme of RAS. The important findings in this study were that Unpurified and purified sample of Aconite significantly decrease renin level in animal model of hypertension. In this 2K1C model due to occlusion of one kidney the GFR was decrease, which cause rise in the level of serum creatinine and BUN in hypertensive model. Both unpurified and purified sample of aconite root caused significant reduction in serum creatinine and BUN in hypertensive model and thus also helps in reduction of blood pressure.

The present study concluded that there was not much more difference in the capacity of reduction of renin-levels, serum creatinine level and BUN by purified and unpurified sample of aconite in hypertensive model, which suggest that antihypertensive effect was not affected due to shodhana process.

Effect of aconite root treatment on oxidative stress markers in kidney tissue.

Oxidative stress markers catalase, superoxide dismutase, malondialdehyde, glutathione levels were measured in kidney tissue homogenate supernatant. The nitric oxide levels are also measured as it is involved in vasodilative effect of a drug.

Renal artery occlusion cause oxidative stress that is responsible for kidney damage and hence hypertension therefore the catalase, glutathione, superoxide dismutase levels are significantly decreased in diseased control group in comparison to normal control group. MDA levels are significantly increased indicating lipid peroxidation in kidney tissue in diseased control group.

It can be seen from the table that aconite root treatment (purified or unpurified form) group there is a significant increase in catalase, glutathione, superoxide dismutase and nitric oxide levels and decrease in MDA levels in kidney tissue when compared with diseased control group (Table IV).

When compared between aconite root treatment groups significant increase was found only in NO levels. It suggests that increase in NO levels may be responsible for protection against pro-arrhythmic activity.

Assessment of antioxidant activities and lipid peroxidation by products in hypertensive

subjects indicate an excessive amount of ROS & a reduction of antioxidant mechanism activity in both blood as well as in several other cellular systems. It is known that reactive oxygen species (ROS) contribute to the pathogenesis of numerous cardiovascular diseases including hypertension, atherosclerosis cardiac hypertrophy and heart failure. NAD(P)H oxidase being the predominant source of ROS (McIntyre and Bohr, 1999). SOD, CAT, and GSH are the three primary antioxidant enzymes among the endogenous systems for removal of reactive oxygen species (Bukan et al., 2003).

Reduction in oxidative stress represents a potential mechanism by which NO prevents arrhythmia. Recently NO mediated reductions in O_2^- have been implicated in preconditioning mediated protection from arrhythmia in dogs. It was found that reductions in arrhythmia were associated with both increased NO production and decreased oxidative stress (Dylan and Qingping, 2011).

CONCLUSION

The unpurified aconite root group showed significant increase in heart rate, increase in QRS complex time and increase in QT interval, however these parameters were statistically insignificant in purified aconite root treated group. The PRA, SC and BUN levels was significantly decreased in aconite root treatment groups. The probable mechanism of antihypertensive activity of aconite root can be attributed to decrease in plasma renin activity, decrease in oxidative stress and increase in NO levels.

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