Analgesic, Anti-inflammatory and neuropharmacological effects of *Atropa belladonna*

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Abstract: The present study was carried out to investigate, *in vivo*, analgesic, anti-inflammatory and neuro-pharmacological activities of the methanolic extract of *Atropa belladonna*. The analgesic activity was measured by acetic acid induced writhing inhibition test. The neuro-pharmacological activities were evaluated by open field, rearing test, cage cross, swim test, head dip and traction tests. The anti-inflammatory activity was assessed by formalin induce inflammation on hind paw. The extract showed highly significant (p<0.001) analgesic activity with % inhibitions of writhing response at doses 100 and 300mg/kg body weight were 28.5% and 57.1%, respectively. The extract at both doses showed significant (p<0.05) sedative effect in-cage cross test and highly significance value (p<0.001) in high dose. In-open field test, the extract showed significant (P<0.05) anxiolytic activity at higher dose whereas in rearing test activity shows significant p-value at both doses. The extract also showed significant value for anti-inflammatory activity. The findings of the study clearly indicated the presence of significant analgesic, neuro-pharmacological and anti-inflammatory properties of the plant, which demands further investigation including, compounds isolation.

Keywords: Analgesic, anxiolytic, anti-inflammatory, *Atropa belladonna.*

INTRODUCTION

Atropa belladonna (Solanaceae) is commonly known as Belladonna or Deadly nightshade, is a perennialherb native to Europe, North Africa and Western Asia. The leaves and berries are very toxic due to tropane alkaloids (Wink & Roberts, 1998).

The common alkaloids of this plant are atropine, scopolamine, and hyoscyamine. They cause a state of delirium when ingested in sufficient amounts (Kuhn *et al*., 2008; Hofmann and Schultes, 1987; Wilson, 2014;Tombs and Silverman, 2004).

A. belladonna is used as muscle relaxant and used for the treatment of muscle spasm in the stomach, intestine and bile duct. It is also used as a cure for peptic ulcers and acts as anesthetic, antidote to some mushrooms poisons, insect bites and nerve gas (Hofmann and Schultes, 1987; Wilson, 2014; Tombs and Silverman, 2004).

Its alkaloid atropine is also a valuable compound due to its ability of relieving Parkinson's disease, asthma, whooping cough, hay fever, and said to regulate heartbeats. Belladonna reduces traumas, paralysis and improves mobility and speech in patients with debilitating disease.

Belladonna is very effective in treatment of eye diseases due to the presence of Atropine that dilates the pupil. Belladonna is externally applied to lessen irritability and pain, and is used as a lotion, plaster or liniment in cases of neuralgia, gout, rheumatism and sciatica. It is good in checking excessive secretions and to alleviate inflammation and to check the sweating of phthisis and other exhausting diseases (Tombs and Silverman, 2004; Wilson, 2014; Hofmann and Schultes, 1987).

Belladonna in small doses combats cardiac palpitation, and the plaster is applied to the cardiac region for the same purpose, alleviating pain and distress.

Belladonna leaves are smoked for treatment of whooping cough and false croup, and when applied externally, they said to relieve skin cancerous symptoms (Wilson, 2014; Hofmann and Schultes, 1987; Tombs and Silverman, 2004).

Belladonna contains the active ingredients atropine and scopolamine, which are anti-cholinergic, meaning that they work by blocking certain nerve impulses involved in the parasympathetic nervous system, which regulates certain involuntary bodily functions or reflexes, including pupil dilation, secretion of glands, and the relaxation of the bronchioles in the lungs, and thereby alleviates the wheezing symptoms of an asthma attack. Caution should be exercised since ingestion of high concentration of atropine can cause severe illness and death (Kuhn *et al*.,

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2008; Hofmann and Schultes, 1987; Wilson, 2014;Tombs and Silverman, 2004).

MATERIAL AND METHOD

Plant materials

The plant was collected from Northern region of Pakistan and identification was made by Dr. Mehjabeen, Dean Faculty of Pharmacy, Federal Urdu University of Arts, Science & Technology, Karachi, Pakistan. After collection a specimen voucher was deposited in the department of Botany, (herbarium voucher # FO-1-1122 2013). It was weighed before drying and after drying also.

Preparation of methanol extract

The collected plant was washed and dried under sun for 10 days. The dried plant was ground into coarse powder with the help of a grinder and was stored in an airtight glass container. The powdered plant material (450g) was extracted by maceration under room temperature using 99.8% (v/v) methanol as solvent through occasional stirring and shaking for 5 days. The extract was then filtered through Whatman filter paper and concentrated at low temperature (40°C) with a rotary evaporator. The dry extract was stored at 4°C until use.

Experimental animals

Albino mice (20-25g weight) were purchased from the Animal House of Dow University of Health Sciences, Karachi and kept under standard laboratory conditions, i.e. (room temperature 25.0±2.0°C, relative humidity 55-65% and 12h light: Dark cycle). They were fed on standard diet and water. The experimental protocols used were approved by the Animal Experimentation Ethics Committee of East West University (Apu *et al*., 2012).

Analgesic activity

The analgesic activity of the extract was evaluated by using acetic acid induced writhing method in mice (Apu *et al*., 2012; Zulfikar *et al*. 2010). According to the method, writhes were induced by intra-peritoneal administration of 0.6% acetic acid solution 30 minutes before to the administration of the extracted substance. They were treated orally with the test substance and number of writhes counted for 30 minutes immediately after acetic acid administration. A reduction in the number of writhing as compared to the Control was considered as evidence for the presence of analgesic and expressed as percentage inhibition of writhing. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing Analgesic activity was expressed as writhing inhibition (%) and was calculated by using the following formula:

Writhing inhibition (%) = $([W_c - W_s]/W_c) \times 100$

Where, W_c is the mean number of writhing of control and W_s is the mean number of writhing of the test sample (Apu *et al*., 2012; Zulfikar *et al*., 2010).

Neuro-pharmacological activities

The methanolic extract was evaluated for its neuro-pharmacological activities by using cage cross method, head dip method, open field, traction test, swimming test, light and dark test, and rearing test.

- Group 1 set of mice 5 were given normal saline for control reading
- Group 2 set of mice 5 were given test drug extract of 100mg dose
- Group 3 set of mice 5 were given test drug extract of 300mg dose

Cage cross test

The experiment was carried out in cage cross apparatus. Each mouse was immediately placed on one side of the specified instrument, after oral administration of test drugs (Subhan *et al*., 2008) the spontaneous movement of the mice from one chamber to other through the hole was observed for 3 minutes. The observation was conducted at 0, 10, 30 and 60 minutes.

Head dip test

The study was conducted using a wooden hole-board apparatus measuring 20cm by 40cm with 16 evenly spaced holes (each of diameter 3cm). 30 minutes after treatment, mice were placed singly on the center of the board and the number of head dip was counted using a tally counter during a 10 minutes trial period (Somani *et al*., 2010).

Open field test

Mice were placed into the open field apparatus and were allowed to explore for 10 minutes and is done with dose of 100mg and 300mg.

Traction test

Mice were kept on traction apparatus to check its learning power and ability to cross it with balance.

Swim test

In swimming box mice was observed for swimming for 6 minutes.

Anti-inflammatory effects

Formalin was used to produce inflammation and anti-inflammation effects were checked by test drug. Formalin was injected into the dorsal surface of a hind paw and the time the animal spent licking the paw was recorded, (Hunskaar *et al*., 1985)**.** Albino rats were used and divided into 6 groups and treated accordingly:

Normal control, formalin induced group (0.1ml/kg),
Formalin+ *Atropa belladonna* (100mg/kg),
Formalin+ *Atropabellodonna* (300mg/kg).

Table 1: Analgesic activity of *A. belladonna*

Treatment	No. of writhes	% Inhibition of writhes
Control	7±1.5	-
AB 100mg	5±1.2	28.5%
AB 300mg	3±1	57.1%
Diclofenac Sodium	4±1.2	42%

Values are expressed as mean SEM ± (n=5); *p<0.005 or **p < 0.0001; compared with control; AB indicates *Atropa belladonna*. Standard drug Diclofenac Sodium is used.

Table 2: Neuro-pharmacological Test of *A. belladonna* extract

	Neuro-pharmacological test	
1) OPEN FIELD		
Treatments	No. of head dips	Level of significance
Control	108±2.84	P<1.000
AB 100mg	89.67±5.95 *	P<0.0195
AB 300mg	95.33±2.86 *	P<0.0103
2) CAGE CROSS		
Treatment	No. of movements	*Level of significance*
Control	25.17±1.33	P<0.0001
AB 100mg	15.17±1.70*	P<0.0009
AB 300mg	6.50±0.76**	P<0.0001
3) REARING TEST		
Treatments	No. of head dips	Level of significance
Control	24.83±2.93	P<1.000
AB 100mg	15.33±0.88 *	P<0.0111
AB 300mg	27.17±1.25 *	P<0.4802
4) TRACTION TEST		
Treatments	No. of head dips	Level of significance
Control	28.83±1.40	P<1.000
AB 100mg	30.33±2.20	P<0.5780
AB 300mg	7.00±0.58**	P<0.0001
5) HEAD DIP TEST		
Treatments	No. of head dips	Level of significance
Control	11.17±0.95	P<1.0000
AB 100mg	6.00±0.73 *	P<0.0015
AB 300mg	6.17±0.60 *	P<0.0012
6) SWIM TEST		
Treatments	No. of head dips	Level of significance
Control	281.83±3.88	P<1.000
AB 100mg	152.17±9.84**	P<0.0001
AB 300mg	190.50±3.78**	P<0.0001

Values are expressed as mean SEM ± (n=5); *p<0.005 or **p<0.0001; compared with control; AB indicates *Atropa belladonna*.

Table 3: Anti-inflammatory activity of *A. belladonna* extract

Treatment	SEM for inflammation time		Level of significance	
	15min	30min	15min	30min
Control	1.23±0.25	1.12±0.10	P<1.0000	P<1.0000
AB 100mg	1.23±0.25	1.23±0.09	P<1.0000	P<0.7071
AB 300mg	1.21±0.22	1.54±0.14	P<0.0039	P<0.0023
Diclofenac sodium	1.09±0.01	0.04±0.01	P<0.0001	P<0.0001

Values are expressed as mean SEM ± (n=5); *p<0.005 or **p<0.0001; compared with control; AB indicates *Atropa belladonna*. Standard drug Diclofenac Sodium is used.

STATISTICAL ANALYSIS

ANOVA for windows was applied for data analysis and statistically analyzed by one-way analysis of variance. Data were presented as mean ± Standard error of the mean. P<0.05 was taken as level of significance. P<0.001 was taken to be the level of highly significance.

RESULTS

In statistically analyzed by one-way analysis of variance. Data were presented as mean ± Standard error of the mean. % Inhibition of writhes for 100mg is 28.5% and 300mg is 57.1% (table 1).

Neuro-pharmacological activity of *A. belladonna* was assessed at 100 and 300mg doses in comparison with the control (table 2).

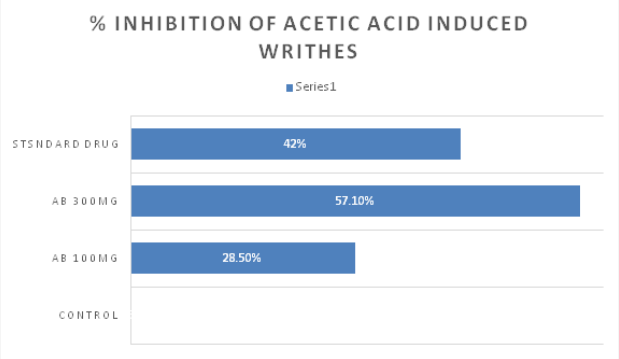

Fig. 1: Dose related analgesic effect of *Atropa belladonna* at dose of 100mg and 300mg. Values are mean ± SD (n=5). Significance difference by ANOVA. Significance value *P*<0.05 shows at dose 100mg and 300mg

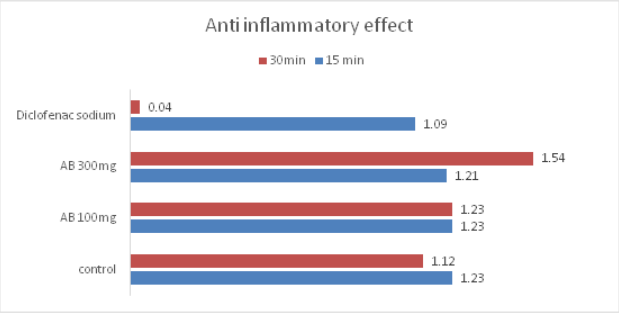

Fig. 2: Dose related anti-inflammatory effect of *Atropa belladonna* at dose of 100mg and 300mg. Values are mean ± SD (n=5). Significance difference was by ANOVA. Significance value *p*<0.05 shows at dose 100mg and 300mg

Anti-inflammatory effect

Table 3 indicates the level of significance at high dose (300mg) of *Atropa belladonna*as compared with the control and standard drug for evaluation of *A. montana* anti-inflammatory effect.

DISCUSSION

Analgesic effects

Historically writhing test have been use to assess analgesic activity of mice. *A. belladonna* analgesic activity was assessed at different doses in comparison with the control. The results obtained were statistically significant at both concentrations of drug. The abdominal cramps induced by acetic acid are due to the activation of pain receptor and prostaglandin. The analgesic effects of *A. belladonna* extract revealed the presence of phyto-chemicals, which require further investigation.

Neuro-pharmacological effects

Open field activity

Open field activity was used to assess the fearfulness or emotional reactivity of rodents, but in our study we found significant result in open field activity at different doses in comparison with control. At high dose of *A. belladonna* extract mice showed high locomotive activity.

Cage cross activity

Cage crossing activity was used to assess the movement activity of mice in cage, but in our study we observed significant result in cage crossing test at different doses in comparison with the control. At high dose of *A. belladonna* extract mice showed significant cage crossing activity.

Rearing test

Rearing test was used to assess the rearing and climbing abilities of mice, but in our study we found significant result in rearing test at different doses in comparison with the control. At high dose of *A. belladonna* extract, mice showed significant rearing activity and low dose of sample showed low activity.

Traction test

Traction test was used to assess the animal's sedative or stimulant abilities of mice by giving this drug in our study we found significant result in traction test at different doses along as compared with the control. At high dose of sample mice showed low stimulant activity.

Head dip test

Head dip test was used to assess the animal's learning activity or abilities of mice by giving this drug in our study we found differences in number of times mice dip head in head dip test at different doses as compared with the control. Both concentrations of drug showed significant activity.

Swim test

Swim test was used to assess the animal's anti-depressant activity of mice. By giving this drug in our study we found significant result in swim test at different doses. As compared with the control, mice showed significant activity at both concentrations of drug.

Formalin test was used to assess analgesic activity of *A. belladonna* extract on administration in mice. In our study we found dose dependent effect in formalin analgesic test as compared to the control. Results showed statistically significant result at higher concentration of drug i.e. 300mg. Analgesic activity may be due to the presence of phytochemicals which blocks the synthesis of prostaglandins. And this anxiolytic activity responds to higher concentration i.e. 300mg.

CONCLUSION

In light of the results of this study, it may be concluded that plant extract of *Atropa belladonna* possesses anti-inflammatory, analgesic and neuro-pharmacological activities, which may be mediated through central mechanism of pain. Study supports the traditional use of plant in pain. It demands further investigation and compound isolation.

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