

Therapeutic Evaluation of Arsenicum Album (C-200 and C-300) Against Trypanosoma Evansi

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Abstract: In continuation from previous preliminary report on anti-trypanosomal activity of Arsenicum album in our fervent search for efficient, effective and affordable therapeutic agents from medicinal plants and other sources against the menacing disease, trypanosomiasis, Arsenicum album pellets (C-200 and C-300) (homeopathic drug) at concentrations ((250-1000 µg mL⁻¹) were screened against Trypanosoma evansi. In this method, two sets of Vero cell line were grown in Dulbecco's Modified Eagle Medium (DMEM) (Sigma) in 96-well flat bottom micro culture plates (Nunc, Denmark). Each well received 100 µl of DMEM containing 5x10⁵ cells/mL. The plates were incubated at 37°C under 5% CO₂ for 48 h to complete development of monolayer. The suspension (100 mL of medium with trypanosomes) was added at rate of 1:1 to test A. album pellets and the ELISA plates were incubated under the same conditions mentioned above. In vitro cytotoxicity test was performed on the same medium at concentrations (1.56-100 µg/mL) but without supplement of foetal calf serum in triplicate and incubated under the same conditions described previously. Results obtained indicated that at 250 µg/mL of A. album (C-200 and C-300), there was significant reductions in trypanosomes count in corresponding ELISA plate wells (40.±0.0 to 1.667±0.33) at 9th h of incubation and but no trypanosome was detected (40.±0.0 to 0.0±0.0 at 9th h in the second concentration, respectively). However, at 500 µg/ml of A. album (C-200), trypanosomes were completely killed at 7th h of incubation that was statically the same as diminazine aceturate, the reference drug at 50 µg/mL. For in vitro cytotoxic test, A. album at both concentrations was non toxic to the Vero cells, while diminazine aceturate was cytotoxic to Vero cells except at concentrations (6. 2.5-1.56 µg/mL). Trypanocidal activity was concentration-time depended. A. album (C-200 and C-300) pellets did possess significant trypanocidal activity, which could be further investigated to understand their maximal potentials.

Keywords: Arsenicum album (C-200 and C-300) (homeopathic drug), in vitro trypanocidal activity, in vivo infectivity test, in vitro cytotoxicity test

I. Introduction

Threat of trypanosomiasis menace has resurged in certain parts of the world, especially, in Africa where it thrives in certain parts of the continent. It is a blood protozoan parasitic disease, which is of zoonotic importance. (1,2). Recently, there have increased reports of its occurrences in new areas invaded by the vectors, reported resistant strains of trypanosomes cum its resistance to the available trypanocides in endemic parts of the world (3).

Efforts in diverse fields of drug discovery are being geared toward development of a new antitrypanosomal drug much from medicinal plants and other sources as well (4, 5, 6, 7, 8, 9).

Trypanosomiasis impacts on livestock production in endemic parts of Africa are enormous with colossal losses (13, 14) and invariably affecting human population at different fronts of life endeavors (1; 2).

It has been documented for a long time that natural products are valuable sources for new drug formulation. Important classes of antimalarial drugs such as quinoline and endoperoxide artemisinin derivatives were originally identified from traditional medicine (15).

Arsenicum album a homeopathy drug, possess antioxidant activity, treatment of skin rashes, treatment of cancer, at different concentrations it has been used in ameliorating chronic arsenic toxicity from repeated sublethal injections of arsenic, reducing cytotoxic effect of arsenic trioxide and supportive evidence of its anticancerous as an alternative medicine against hepatocarcinogenesis all in mice (*Mus musculus*) (16, 17, 18).

Chemotherapeutic agents in used against trypanosomiasis have been developed more than half a century on, and are beset with changelings such as problems like, high cost, toxicity, and unavailability in certain parts of the world where this disease thrives with huge attendance catastrophes (1, 5).

Emergent of resistant strain of trypanosomes and resistance to the available trypanocides at distinct strata have been documented in endemic regions of the world where it possess huge obstacle to treatment of clinical cases and for prophylactic purpose (5, 19).

As a result of aforementioned obstacles to effective and efficient chemotherapeutic approach to trypanosomiasis, *Arsenicum album* (C-200 and C-300) potentized homeopathic drug of different concentrations were evaluated against *Trypanosoma evansi*.

II. Aim And Objectives

This research work is aimed at discovery a potent candidate compound from no medicinal plant source that could be used by pharmaceutical company in near future for the production of a new effective, efficient and affordable trypanocide against both animals and humans trypanosomiasis.

The objectives of this study are to screen *Arsenicum album* (C-200 and C-300) homeopathic drug against *Trypanosoma evansi* for its trypanocidal activity and *in vitro* cytotoxicity effects.

III. Material And Methods

Arsenicum album

Arsenicum album (homeopathic drug) (C-200 and C-300) pellets were obtained from standard pharmaceutical chemist in Bareilly, Uttar Pradesh, (UP), and subsequently identified by the relevant authority of Indian Veterinary Research Institute, Izatnagar-Bareilly, India.

In vitro trypanocidal activity

It was carried out with modified method of Oliveira et al., (20). A Vero cell line (SIGMA) was grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 20-40% foetal calf serum (FCS), GIBCO USA and antibiotics (100 iu penicillin, 100 µg streptomycin and 40 µg gentamycin) in 96-wells flat bottom microculture plates (NUNC, Denmark). Each well received 100 µl of DMEM containing 5x10⁵ cells mL⁻¹. Plates were incubated at 37°C under 5% CO₂ for 12 h. After the formation of confluent monolayer, the medium was discarded and replaced with a fresh one. Finally, a high parasitaemic blood from mouse was diluted with DMEM to obtain 1x10⁶ parasites mL⁻¹. Suspension (100 ml of medium with trypanosomes) was added at the rate of 1:1 to test *A. album* concentrations (C-200 and C-300) and the plates were incubated under the same conditions mentioned above. The test was repeated at least thrice. 1% DMSO in distilled water was used as control (21).

In vivo infectivity assessment

Following successful anti-trypanosomal activity test, contents of microculture plate wells with reduced and apparently killed trypanosomes by *A. album* pellets at distinct concentrations were inoculated (0.1ml mouse-1) into two groups of mice (six group-1) via intra-peritoneal route, and observed for more than 30 days for parasitaemia (22, 23).

In vitro cytotoxicity test

Cytotoxic effects of the *A. album* (C-200 and C-300) pellets were determined according to the method described by Sidwell and Hoffman (24). Vero cell line was grown in Dulbecco's Modified Eagle Medium (DMEM)(Sigma) Gibco, USA antibiotics (100 units penicillin, 100 µg streptomycin and 40 µg gentamycin) in 96-well flat bottom microculture plates (Nunc, Denmark). Each well received 100 µL of DMEM containing 5x10⁵ cells/mL. The plates were incubated at 37 °C under 5% CO₂ for 48 h. After the formation of confluent monolayer, the medium was discarded and replaced with a fresh one. A high parasitaemic blood from mouse was diluted with DMEM to obtain a final parasite of 1x10⁶ parasites/mL. Confluent monolayer of Vero cell was treated with serial dilutions of test *A. album* (C-200 and C-300) (1.56-100 µg/mL) in triplicate and incubated under the same conditions described previously. After 24 h of incubation, the culture plates were observed for evidence of cytotoxic effects. The plates were incubated for 72 h and observed daily. It was repeated thrice. In each case, after the 72 h of incubation, the culture media of the incubated Vero cells were discarded. The adhered cells were stained with a drop of crystal violet in phosphate buffered solution. The plate was incubated for 24 hours at 37°C in an ordinary incubator. After 24 h of incubation, the culture plate was observed for evidence of cytotoxic effects.

Statistical analysis

Results of trypanocidal activity were expressed as mean ± SEM. Statistical significance was determined by Sigma Stat (Jandel), USA.

IV. Results

In vitro trypanocidal activity

Therapeutic activities of *A. album* (C-200 and C-300) pellets against *Trypanosoma evansi* were as contained in Tables (1 and 2). Trypanocidal activity varied from immobilization, reduction and to the killing of

trypanosomes at different concentrations used. Results indicated that at 250 µg/mL of *A. Album* (C-200 and 300) concentrations, there was significant reductions in trypanosomes count in corresponding ELISA plate wells (40.±0.0 to 1.667±0.33) at 9th h of incubation and no trypanosome was detected (40.±0.0 to 0.0±0.0) in the second micro culture plates. However, at 500 µg/ml of *A. album* (C-200), trypanosomes were completely killed at 7th h of incubation that was statically the same as diminazine aceturate, the reference drug, at 50 µg/mL. An average mean trypanosomes count of 37.67±0.58 is statistically critical value. Average mean trypanosomes count from 37.67±0.58 and below was significant between the treatment groups and negative control ($p \leq 0.05$ to 0.01).

In vivo infectivity test

Two sets of mice groups (A and B) inoculated with contents of ELISA plate wells with completely killed trypanosomes count (40.00±0.0 to 0.0±0.00) from *A. album* (C-200 and C-300) concentrations survived for more than 30 days. Those in groups (C and D) inoculated with contents of ELISA plate wells with reduced trypanosomes count died of parasitaemia.

In vitro cytotoxicity test

A. *Album* (C-200 and C-300) pellets were non cytotoxic to Vero cells. But diminazine aceturate, standard reference drug, was cytotoxic to Vero cells except at 12.5-1.56 µg/mL (Tables 3 and 4).

V. Discussion

In this current investigation, *A. album* (C-200 and C-300) pellets had demonstrated its varied degrees of therapeutic activity against *Trypanosoma evansi* at different concentrations. The striking and surprised finding was non cytotoxic effect on Vero cells that gives it an edge over diminazine, aceturate, the standard reference drug, which gives a fillip for further research.

In vitro trypanocidal activity

Therapeutic activity of *A. album* (C-200 and C-300) pellets against *T. evansi* is in line with *in vitro* trypanocidal activity of *A. album* (C-30) where trypanosomes were drastically reduced at concentrations (250-750 µg/mL) and completely killed at 1000 µg/mL in corresponding ELISA plate wells., *in vitro* trypanocidal activity of methanolic extracts of *Quercus borealis* leaves and *Zingiber officinale* roots with complete killing of trypanosomes at 500 and 750 µg/mL, trypanocidal activity of methatnolic extracts (50 and 100%) of *Emblica officinalis* dried fruits and therapeutic activity of partial purified fractions of *Emblica officinalis* dried fruits against *T. evansi* with varied degrees of trypanocidal activity (10, 27, 8, 3, 12).

The mechanism of action could be due to intercalation of *A. album* pellets with DNA of the trypanosomes, which often leads to its death as documented in the research outcomes done with extracts, fractions and isolated compounds from medicinal plants (10, 5, 6).

In vivo infectivity test

In vivo infectivity assessment of therapeutic activity of *A. album* (C-200 and C-300) is comparable to trypanocidal activity of *A. album* (C-30), *in vitro* and *in vivo* anti-trypanosoml activity of *Terminalia chebula* dried fruits, trypanocidal activity of 50% methaolic tree bark of *Khaya senegalensis* and therapeutic activity of partially purified fractions of *E. officinalis* dried fruits where inoculated mice with contents of ELISA plate wells with apparently killed trypanosomes survived (10,11, 23, 8 , 12).

In vitro cytotoxicity test

A. *In vitro* cytotoxicity tests on Vero cells are amazing in term of its level of toxicity. These results are comparable to trypanocidal activity of *A. album* (C-30), *in vitro* and *in vivo* anti-trypanosomal activity of *Terminalia chebula* dried fruits, therapeutic activity of partially purified fractions of *E. officinalis* dried fruits, *in vitro* cytotoxicity test of *Camellia sinensis* leaves, methanolic extract of *Vitex negundo* leaves, trypanocidal activity of methanolic extracts (50 and 100%) of *Emblica officinalis* dried fruits and antitrypanosomal activity of *Picrorrhiza kurroa* rhizomes against *Trypanosoma evansi*, in which similar no cytotoxic effect was observed at certain ranged of concentrations as glaringly noted in the current investigation (10, 11, 12, 25, 29, 30). *A. album* (C-200 and C-300) pellets were non toxic to Vero cells in comparison to diminazine aceturate, the reference drug often used for both therapeutic and prophylactic cases against trypanosomes.

VI. Conclusion

In the current investigation on anti-trypanosomal from non medicinal source, it could be concluded that *Arsenicum album* (C-200 and C- 300) potentized pellets did exhibit varied degrees of therapeutic activity

against *T. evansi* as per their inherent possession of such compound(s) responsible for the activity. These results are significant in comparison to the earlier preliminary report of trypanocidal activity of *A. album* (C-30) in which moderate trypanocidal activity was documented. *A. album* (C-200 and C-300) non toxic to the Vero cells as observed gives it's an advantage over diminazine aceturate in cytotoxicity tests. Additional tailor researches need to be carried out on *A. album* pellets to put it in a commanding lead for a potential candidate for anti-trypanosomal compound (s) that may pave way for a new trypanocide.

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Table 1. *In vitro* trypanocidal activity of *Arsenicum album* tincture (C- 200) against *Trypanosma evansi* on Vero cell line

Concentration of pellets in µg/ml	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h
250	33.00±0.58	28.33±0.33	23.00±0.58	16.67±0.67	13.33±0.88	10.67±0.67	8.667±0.67	4.667±0.67	1.667±0.33
500	29.33±0.33	23.67±0.33	17.00±0.0	12.33±0.33	8.333±0.33	3.333±0.33	1.667±0.33	0.0±0.0	0.0±0.0
750	27.67±0.33	22.33±0.33	13.33±0.33	7.667±0.33	1.000±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1000	24.67±0.33	13.33±0.33	8.333±0.33	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Diminazine aceturate (50)	23.33±0.88	9.333±0.33	1.333±0.33	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Positive control									
Control (Negative control)	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0

Bioassay status: significant reduction of parasites counts from concentration of 250 µg/ml and complete killing of parasites at 500 µg/ml at 7th hour of observation. An average mean trypanosomes count of 37.67± 0.58 is statistically critical value. Average mean from 37.67± 0.58 and below is significant between the treatment groups and negative control. (P ≤ 0.05 to 0.01).

Table 2. *In vitro* trypanocidal activity of *Arsenicum album* tincture (C- 300) against *Trypanosma evansi* on Vero cell line

Concentration of pellets in µg/ml	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h
250	32.33±0.67	27.33±0.33	21.33±0.33	14.67±0.67	11.00±0.58	8.000±0.67	4.000±0.58	1.667±0.33	0.0±0.0
500	22.67±4.8	21.67±0.33	13.33±0.33	9.333±0.33	3.333±0.33	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
750	25.67±0.67	20.33±0.33	12.33±0.33	5.000±0.58	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1000	21.67±0.33	11.67±0.33	5.000±0.58	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Diminazine aceturate (50)	23.33±0.88	9.333±0.33	1.333±0.33	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Positive control									
Control (Negative control)	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0	40.00±0.0

Bioassay status: significant reduction of parasites counts from concentration of 250 µg/ml and complete killing of parasites at the same concentration at 9th hour of observation. An average mean trypanosomes count of 37.67± 0.58 is statistically critical value. Average mean from 37.67± 0.58 and below is significant between the treatment groups and negative control. (P ≤ 0.05 to 0.01).

Table 3. Cytotoxic effect of pellets of *Arsenicum album* (C-200), a homeopathic drug on Vero cell line compared to diminazine aceturate (Berenil)

Concentration of test material in µg/ml	Effects of <i>Arsenicum album</i> (200, tincture) at various periods of incubation (24 h, 48 h, 72 h)						
	<i>Arsenicum album</i>	Berenil	<i>Arsenicum album</i>	Berenil	<i>Arsenicum album</i>	Berenil	Control
100	0	66.6%	0	100%	0	100%	0
50	0	33.3%	0	100%	0	100%	0
25	0	0	0	100%	0	100%	0
12.5	0	0	0	0	0	33.3%	0
6.25	0	0	0	0	0	0	0
3.13	0	0	0	0	0	0	0
1.56	0	0	0	0	0	0	0

Arsenicum album was non toxic to Vero cell line while, dimainazine aceturate did except at concentrations range of 6.25-1.56 µg/ml. Same concentrations were used for diminazine aceturate (Berenil)

Table 4. Cytotoxic effect of pellets of *Arsenicum album* (C-300), a homeopathic drug on Vero cell line compared to diminazine acetate (Berenil)

Concentration of test material in µg/ml	Effects of <i>Arsenicum album</i> (300, tincture) at various periods of incubation (24 h, 48 h, 72 h)						
	<i>Arsenicum album</i>	Berenil	<i>Arsenicum album</i>	Berenil	<i>Arsenicum album</i>	Berenil	Control
100	0	100%	0	100%	0	100%	0
50	0	66.6%	0	100%	0	100%	0
25	0	0	0	100%	0	100%	0
12.5	0	0	0	0	0	66.6%	0
6.25	0	0	0	0	0	0	0
3.13	0	0	0	0	0	0	0
1.56	0	0	0	0	0	0	0

Arsenicum album was non toxic to Vero cell line while, diminazine acetate did except at concentrations range of 6.25-1.56 µg/ml.

Same concentrations were used for diminazine acetate (Berenil)