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Trypanocidal Activity of Arsenicum Album (C-30) against Trypanosoma Evansi

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ABSTRACT: *In quest of a new drug for the treatment of trypanosomosis, a zoonotic disease, Arsenicum album tincture (C-30) (homeopathic drug) at concentrations ((250-1000 µg mL-1) was screened against Trypanosoma evansi. In this method, a Vero cell line was 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 tincture and the ELISA plate was incubated under the same conditions mentioned above. In vitro cytotoxicity 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 showed that at 250 µg/mL of A. Album, there was marked reduction in trypanosomes count (40.±0.0 to 22.33±0.33) at 9 h of incubation. There was drastic reduction of trypanosomes count at concentration of 750 µg/mL ((40.±0.0 to 1.667±0.33). But, at 1000 µg/mL of A. album, trypanosomes were not detected in the corresponding ELISA plate wells at 7 h of incubation (40.±0.0 to 0.0±0.0) that was statically the same as diminazine aceturate, the reference drug, at 50 µg/mL. A. album and diminazine aceturate were cytotoxic to Vero cells except at 12.5-1.56 and 25-1.56 µg/mL. A. album (C-30) was 50% less cytotoxic in comparison to diminazine aceturate. For in vivo trypanocidal activity, mice treated with A. album at 200 mg/kg body weight died at day 8 post infected. A. album prolonged the survival day of the mice but could not cure them. Trypanocidal activity was concentration-time depended. A. album (C-30) did possess moderate trypanocidal activity, which could be further research on to obtain its full potential.*

Keywords: *Arsenicum album (C-30) (homeopathic drug), in vitro and in vivo trypanocidal activity, in vivo infectivity test, in vitro cytotoxicity*

I. Introduction

Trypanosoma evansi is one of the causative agents of trypanosomosis, a blood protozoan disease that 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).

Currently, concerted efforts at all levels are needed to develop new drugs via identification and isolation of trypanocidal compounds from medicinal plants and other sources available (4. 5, 6.'7, 8 9).

Livestock production has been seriously affected in those areas where trypanosomosis is endemic (10, 11) and invariably affecting human population with catastrophic outcome (1; 2).

Since time immemorial, natural products are valuable sources for new drug formulation. Important classes of antimalarial drugs such as quinoline and endoperoxide atermisinin derivatives were originally identified from traditional medicine (12).

Arsenicum album (C-30), a homeopathy drug, at different concentrations have been used in ameralioting chronic arsenic toxicity from repeated sublethal injections of arsenic, reducing cytutoxic effect of arsenic trioxide and supportive evidence of its anticancerous as an alternative medicine against hepatocarcinogenesis all in mice (*Mus musculus*) (13, 14, 15).

Current drugs in used against trypanosomosis have been developed more than half a century on, and they are bedeviled with problems like, high cost, toxicity, and unavailability in certain parts of the world where this disease strives with huge attendance catastrophes (1, 5).

Resistances to the available trypanocides have been reported (5, 16).

Hence, this underscore this research work and to checkmate the menace of trypanosomosis.

II. Aim And Objectives

The aim of this research is to discover a potent candidate compound that could be used for the production of a new effective, efficient and affordable trypanocide against both animals and humans trypanosomosis.
The objective of this study is to screen *Arsenicum album* (C-30) homeopathic drug against *Trypanosoma evansi* for its anti-trypanosomal activity and *in vitro* cytotoxicity effects.

III. Material And Methods

Arsenicum album

Arsenicum album (homeopathic drug) (C-30) pellets was obtained from standard pharmaceutical chemist in Bareilly, Uttar Pradesh, (UP), and subsequently identified by the authority of Indian Veterinary Research Institute, Izatnagar-Bareily, India.

In vitro tryponocidal activity

It was done with modified method of Oliveira et al., (17). 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 37oC 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* and the plate was incubated under the same conditions mentioned above. The test was repeated at least thrice.
Stock of test *A. album* (C-30) was solubilized in 1% dimethylsuphoxide (DMSO). The concentration in the experiment had no deleterious effect by itself on host cells or parasites. 1% DMSO in distilled water was used as control (18).

In vivo infectivity assessment

After incubation for anti-trypanosomal activity was completed, contents of microculture plate wells with reduced and apparently killed trypanosomes by *A. album* pellets 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 (19, 20).

***In vitro* cytotoxicity test**

Cytotoxic effects of the *A. album* (C-30) pellets were determined according to the method described by Sidwell and Hoffman (21). 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% CO_2 for 48h. 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 $1x10^6$ parasites/mL. Confluent monolayer of Vero cell was treated with serial dilutions of test *A. album* (C-30) (1.56-100 µg/mL) in triplicate and incubated under the same conditions described previously. After 24 h of incubation, the culture plate was observed for evidence of cytotoxic effects. The plate was 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.
In vivo trypanocidal activity of *Arsenicum album*. Pellets (C-30)
In vivo test was carried out in according to the method of Freiburghaus et al., (22). In this method, six mice in a group were inoculated with trypanosomes (1×10^4 /ml). Infected mice were treated with *A. album* at concentrations (12.5-200 mg/kg body weight) intraperitoneally 48 h post on set of parasitemia. 1% of DMSO was added to *A. album* (C-30) diluted with DMEM. A drop of blood was taken from the tail-end of the mice daily and parasites were counted as previously described**.**

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

Anti-trypanosomal activity of *A. album* (C-30) pellets is contained in Table 1. Anti-trypanosomal activity varied from immobilization, reduction and to the killing of trypanosomes at different concentrations used. Results showed that at 250 µg/mL of *A. album*, there was reduction in trypanosomes count in corresponding ELISA plate wells but no complete killing of trypanosomes (40.±0.0 to 22.33±0.33). There was drastic reduction of trypanosomes count at concentration of 750 µg/ml ((40.±0.0 to 1.667±0.33). However, at 1000 µg/ml of *A. album* (C-30), trypanosomes were not detected in the corresponding ELISA plate wells at 7 h of incubation (40.±0.0 to 0.0±0.0) 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

Mice in group A inoculated with contents of ELISA plate wells with completely killed trypanosomes count (40.00±0.0 to 0.0±0.00) at 1000 µg/mL of *A. album* survived for more than 30 days, while those in group B inoculated with contents of ELISA plate wells with reduced trypanosomes count (40.00±0.0 to 1.33±0.33) at 750 µg/mL died of parasitaemia.

In vitro cytotoxicity test

A. album (C-30) pellets and diminazine aceturate were cytotoxic to Vero cells except at 25-1.56 and 12.5-1.56 µg/mL. *A. album* (C-30) was 50% less toxic than diminazine aceturate on Vero cells, the reference drug often used for both therapeutic and prophylactic cases against trypanosomes.

In vivo trypanocidal activity of *Arsenicum album*. Pellets (C-30)

At 12.5 and 25 mg/kg body weight of *A. album*, mice in these groups died at day 6 post infection. But at 50, 100 and 200 mg/kg body weight of *A. album*, mice in the respective groups died at day 7 and 8. Group of mice treated with *A. album* at dose rate of 200 mg/kg had the best result in term of trypanosomes counts. The trypanocidal activity was concentration- time depended manner.

V. Discussion

In respect to *A. album* (C-30) pellets, though it possess inherent toxicity of its own, the *in vitro* trypanocidal activity and corresponding level of *in vitro* toxicity indicates an encouraging results. This invariably, put its apar with some previously screened medicinal plants.

In vitro trypanocidal activity

Anti-trypanosomal activity of *A. album* (C-30) pellets is comparable to *in vitro* trypanocidal activity of methanolic plant extracts (MPES) of medicinal plants used in treatment of trypanosomosis in northern Nigeria at an effective concentration of 8.3 mg mL-1 (23), *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 and trypanocidal activity of methatnolic extracts (50 and 100%) of *Emblica officinalis* dried fruits (24, 8).

The mechanism of trypanocisal activity of *A. album* is yet to be determined. Perhaps, it might 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 (5, 6)..

In vivo infectivity Test

In vivo infectivity assessment of anti-trypanosomal activity of *A. album* is comparable to anti-trypanosomal effect of the aqueous extract of methanolic plant extract (MPE) of *Terminalia chebula* dried fruits and trypanocidal activity of 50% methaolic tree bark of *Khaya senegalensis* where inoculated mice with contents of ELISA plate wells with apparently killed trypanosomes survived (20, 8).

In vitro cytotoxicity Test

In vitro cytotoxicity test on Vero cells is quite interesting as per its level of toxicity. This result is in line with *in vitro* cytotoxicity tests of *Camellia sinensis* leaves, methanolic extract of *Vitex negundo* leaves, trypanocidal activity of methanolic extracts (50 and 100%) of *Emblica officinalis* dried fruits and antitripanosomal activity of *Picrorrhiza kurroa* rhizomes against *Trypanosoma evansi*, in which similar cytotoxic effects such as distortion, swelling,

sloughing and death of Vero cells compared to negative normal cells in control ELISA wells were observed 25, 26, 27.9).

In vivo trypanocidal activity of *Arsenicum album*. Pellets (C-30)

*In vivo t*rypanocidal activity of *A. alum* is in line with trypanocidal activity of methanolic leaf extract of *Camellia sinensis* and antihepatotoxic, and antitrypanosomal activity of *Nuclear latifolia* root bark in which mice in distinct groups died at different days post infection. Though mice lives were prolonged neither could not cure them. (26, 28)

VI. Conclusion

From this preliminary investigation so far of *A. album* (C-30) potentized pellets in *in vitro* anti-trypanosomal activity, it could be concluded that it possesses moderate trypanocidal activity and much lesser cytotoxicity effects in comparison to diminazine aceturate, the standard reference drug used in clinical cases and prophylactic as well. Perhaps, higher concentrations of *A. album* will give better results than this. Further investigations, in laboratory animals, are required to unveil its full potential as a candidate for antitrypanosomal drug in near future.

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Conflict of interests: There is no conflict of interest in respect to publication of this research outcome.

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Table I. *In vitro* trypanocidal activity of *Arsenicum album* pellets (C- 30) against *Trypanosma evansi* on Vero cell line

. Concentration of test material in mg/kg body weight	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
12.5	6.500±0.43	10.17±0.75	28.50±0.27	39.65±0.356	-	-	-
25	6.667±0.33	9.67±0.49	28.50±0.54	37.00±0.01	-	-	-
50	6.667±0.33	7.67±0.33	16.33±0.62	24.17±0.56	35.50±0.63	-	-
100	6.500±0.34	6.83±0.48	12.67±0.42	22.33±0.88	32.53±0.98	42.57±0.20	-
200	6.500±0.42	61.50±0.55	11.67±0.42	19.00±0.96	28.00±0.96	37.78±0.33	-
Diminazine aceturate (10) Positive control	6.167±0.31	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Control (Negative control)	6/832 ± 0.32	13.50±0.56	39.50±0.43	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Bioassay status: significant reduction of parasites counts from concentration of 750 ug/ml and complete killing of parasites at 1000 ug/ml at 7th hour of observation. An average mean parasites 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 ll. Cytotoxic effect of Arsenicum album (30) pellets, a homeopathic drug, on Vero cell line compared to diminazine aceturate (Berenil)

Concentration of test material in µg/ml	. Effects of pellets *Arsenicum album* (30, pellets) at various periods of incubation (24 h, 48 h, 72 h)						
	Arsenicum album	Berenil	*Arsenicum album*	Berenil	*Arsenicum album*	Berenil	Control
100	33.3%	66.6%	100%	100%	100%	100%	0
50	33.3%	33.3%	33.3%	100%	33.3%	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 and dimainazin e aceturate were toxic to Vero cell line except at concentrations range of25- 1.56 and 6.25-1.56 µg/ml.
Same concentrations were used for diminazen aceturate (Berenil)

Table lll. In vivo trypanocidal activity of Arsenicum album. Pellets (C-30).

. Concentration of test material in mg/kg body weight	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
12.5	6.500±0.43	10.17±0.75	28.50±0.27	39.65±0.356	-	-	-
25	6.667±0.33	9.67±0.49	28.50±0.54	37.00±0.01	-	-	-
50	6.667±0.33	7.67±0.33	16.33±0.62	24.17±0.56	35.50±0.63	-	-
100	6.500±0.34	6.83±0.48	12.67±0.42	22.33±0.88	32.53±0.98	42.57±0.20	-
200	6.500±0.42	61.50±0.55	11.67±0.42	19.00±0.96	28.00±0.96	37.78±0.33	-
Diminazine aceturate (10) Positive control	6.167±0.31	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Control (Negative control)	6/832 ± 0.32	13.50±0.56	39.50±0.43	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

At dose rate of 200 mg/kg body weight, the mice in this group survived for 6 days post on set of parasitaemia. There was degree of significant difference between treated groups with test material in comparison to negative control that survived for only 3 days ($P \leq 0.05$ to 0.01)

Table I. ***In vitro*** **trypanocidal activity of** ***Arsenicum album*** **pellets (C- 30) 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	37.33±0.33	36.67±0.33	34.67±0.33	32.33±0.33	30.33±0.33	28.33±0.33	26.33±0.33	24.33±0.33	22.33±0.33
500	35.00±0.58	32.67±0.67	30.33±0.33	28.33±0.33	26.33±0.33	23.67±0.33	19.67±0.33	16.33±0.33	12.67±0.67
750	32.67±0.33	29.33±0.33	25.33±0.33	22.67±0.67	19.33±0.33	13.33±0.33	10.33±0.33	7.000±0.58	1.667±0.33
1000	29.00±0.0	24.33±0.33	19.67±0.33	12.33±0.33	8.333±0.88	2.667±0.67	0.0±0.0	0.0±0.0	0.0±0.0
Diminazine aceturate (50) Positive control	22.33±0.33	9.333±0.67	1.000±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
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 750 µg/ml and complete killing of parasites at 1000 µg/ml at 7th hour of observation. An average mean parasites 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. ($P \leq 0.05$ to 0.01).

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	Arsenicum album	Berenil	*Arsenicum album*	Berenil	*Arsenicum album*	Berenil	Control
100	33.3%	66.6%	100%	100%	100%	100%	0
50	33.3%	33.3%	33.3%	100%	33.3%	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 and dimainazin e aceturate were toxic to Vero cell line except at concentrations range of25- 1.56 and 6.25-1.56 µg/ml.

Same concentrations were used for diminazen aceturate (Berenil)

Table lll. ***In vivo trypanocidal activity of Arsenicum album. Pellets (C-30).***

Concentration of test material in mg/kg body weight	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
12.5	6.500±0.43	10.17±0.75	28.50±0.27	39.65±0.356	-	-	-
25	6.667±0.33	9.67±0.49	28.50±0.54	37.00±0.01	-	-	-
50	6.667±0.33	7.67±0.33	16.33±0.62	24.17±0.56	35.50±0.63	-	-
100	6.500±0.34	6.83±0.48	12.67±0.42	22.33±0.88	32.53±0.98	42.57±0.20	-
200	6.500±0.42	61.50±0.55	11.67±0.42	19.00±0.96	28.00±0.96	37.78±0.33	-
Diminazine aceturate (10) Positive control	6.167±0.31	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Control (Negative control)	6/832 ± 0.32	13.50±0.56	39.50±0.43	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

At dose rate of 200 mg/kg body weight, the mice in this group survived for 6 days post on set of parasitaemia. There was degree of significant difference between treated groups with test material in comparison to negative control that survived for only 3 days ($P \leq 0.05$ to 0.01).