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Anticonvulsant activity of *Argentum metallicum*, a homeopathic preparation

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ABSTRACT

Aim of present investigation was to evaluate anticonvulsant profile of Argentum Metallicum a homeopathic preparation in various models of convulsion by assessing various behavioral and biochemical parameters in laboratory animals. Anticonvulsant activity of Argentum Metallicum (30 CH, 200 CH and 1 M) was evaluated against pentylenetetrazole (PTZ), picrotoxin (PTX), strychnine (STR), isoniazid (INH) and maximal electroshock (MES) induced convulsions in mice as well as electrical kindling model in rats. The various in-vitro parameters were also determined including brain gamma amino butyric acid (GABA), nitric oxide (NO) and xanthine oxidase (XO). Diazepam and Phenytoin were used as reference anticonvulsant drugs for comparison. A single intraperitoneal injection of PTZ (90mg/kg), STR (5 mg/kg) and Isoniazid (300 mg/kg) and subcutaneous injection of PTX (3.5 mg/kg) resulted in hind-limb, tonic-clonic convulsion along with lethality in mice, whereas twice daily auricular stimulation resulted progressive severity of seizures in rats. It also significantly decreased ($P < 0.001$) brain GABA level in PTZ, PTX and INH model as well as significantly elevated ($P < 0.001$) brain NO and XO level in PTZ, PTX, INH as well as STR model. Mice treated with Argentum Metallicum (200 CH and 1 M) delayed onset of convulsion along with duration of tonic-clonic convulsions. It also significantly reduced mortality in mice. Rats treated with Argentum Metallicum (200 CH and 1 M) showed significant and dose dependant ($P < 0.05$ and $P < 0.001$) amelioration in severity of electrically kindled seizures and total number of rats seizure per group. Treatment with Argentum Metallicum (200 CH and 1 M) significantly ($P < 0.01$ and $P < 0.001$) elevated the brain GABA level and decreased brain NO and XO level in dose dependant manner. Argentum Metallicum exhibits its antiepileptic activity through GABAegic mechanism and by modulation of endogenous antioxidants like NO and XO.

Keyword: Anticonvulsant, *Argentum Metallicum*, Brain GABA, Homeopathy, Nitric oxide, Xanthine oxidase.

INTRODUCTION

Epilepsy is a neurological disease affecting 8.8 individual per thousand of population. Over a period of time it was observed that focusing the health strategy on communicable diseases seldom proved effective. Rise in better nutrition and social welfare programs the paradigm now shifted to the non communicable neurological disorders [1]. Abnormal neuronal firing and excitatory neurotransmitter play pivotal role in precipitation of seizures in patients [2, 3].

Antiepileptic drug therapy is clinically effective but the adjuvant adverse effect (drowsiness, dizziness, nausea, irritability and hyperactivity) put the blemish on the pharmacotherapeutic profile [4]. At such a critical juncture, medical decision maker is left stranded with lack of optimum treatment algorithms.

Epilepsy induced by the systemic administration of chemicals like pentylenetetrazole (PTZ), picrotoxin (PTX), strychnine (STR) and isoniazid (INH) are reproducible laboratory animal model for preclinical evaluation of potential drug for epilepsy [5-7].

Homeopathy is a popular alternative mode of treatment and provides a ray of hope as the side effect profile is minimum and the efficacy is proven clinically over a century [8-10].

Anticipating better efficacy of homeopathic medicines in epilepsy already depends on the systematic unprejudiced drug proving on healthy human organisms; which in turn is applied as therapeutics [11]. An array of homeopathic medicines including Absinthium, Artemisia vulgaris, Silicea, Calcarea arsenica, Belladonna, etc. have been proven for management of epilepsy clinically [12, 13] and preclinically [14, 15]. From the bulk of such clinically proven medicines *Argentum Metallicum* showed a congruent symptom similarity for implication of the drug in laboratory animal models of epilepsy to elucidate the possible way of objectively decide mechanism. The homeopathic remedy *Argentum Metallicum* is made from Metallic Silver. *Argentum Metallicum* is well documented in literature to have antianxiety, antipsoriatic and antipsychotic activity [16]. However, there is dearth of pharmacological credence to prove its mettle.

Hence, the objective of present investigation was to evaluate anticonvulsant profile of *Argentum Metallicum* in various models of convulsion by assessing various behavioral and biochemical parameters.

MATERIAL AND METHODS

1.1. Animals:

Adult male Swiss albino mice (18-22 g) and male Wistar rats (180-200 g) were purchased from National Institute of Biosciences, Pune and housed in quarantine for one week at the institute animal house separately in groups of six animals per cage at standard laboratory conditions. Animals had free access to food (standard chaw pellet, Pranav Agro industries Ltd., Sangli, India) and water ad libitum. Research protocol was approved by the Institutional Animal Ethics Committee and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

1.2. Drugs and solutions:

Argentum Metallicum (AM) (KR Homeo Pharmacy, Pune), Pentylenetetrazole (PTZ), Strychnine (STR) (Sigma Aldrich India), Phenytoin (PHY) (Eptoin®, Sun Pharma Ltd., India), Diazepam (DZP) (Calmpose®, Ranbaxy Ltd., India) and Isoniazid (INH) (Solonex®, Macleods, Mumbai, India) were used in present study. All other reagents were purchased from S.D. Fine Chemicals, Mumbai, India.

1.3. Assessment of anticonvulsant activity:

1.3.1. Pentylenetetrazole (PTZ) induced convulsions:

The mice were randomly divided into six groups containing six mice in each group as follows:

Group I: *Argentum Metallicum (30 CH, 0.4 ml, i.p.);*
Group II: *Argentum Metallicum (200 CH, 0.4 ml, i.p.);*
Group III: *Argentum Metallicum (1 M, 0.4 ml, i.p.);*
Group IV: *Diazepam (5 mg/kg, i.p.);*
Group V: *Vehicle control (dispensing alcohol, 0.4 ml, i.p.).*
Group VI: *Distilled water (0.4 ml, i.p.)*

The induction of PTZ induced convulsion was carried out according to previously described method [17]. Immediately after PTZ administration mice were observed for next 30 min for following symptoms:

- Onset of convulsion, duration of clonic convulsion, duration of tonic convulsion
- incidence (number of mice showing convulsions); mortality

1.3.2. Picrotoxin (PTX) induced convulsions:
The mice were randomly divided into groups as described above. The induction of PTX induced convulsion was carried out according to previously described method [18]. Immediately after PTX administration mice were observed for next 30 min for following symptoms:

- Onset of convulsion, duration of clonic convulsion, duration of tonic convulsion
- incidence (number of mice showing convulsions); mortality

1.3.3. Strychnine (STR) induced convulsions:
The mice were randomly divided into groups as described above. The induction of STR induced convulsion was carried out according to previously described method [19]. Immediately after STR administration mice were observed for next 60 min for following symptoms:

- Onset of convulsion, duration of clonic convulsion, duration of tonic convulsion
- incidence (number of mice showing convulsions); mortality

1.3.4. Isoniazid (INH) induced convulsions:
The mice were randomly divided into groups as described above. The induction of INH induced convulsion was carried out according to previously described method [18]. Immediately after INH administration mice were observed for next 120 min for following symptoms:

- Onset of convulsion, duration of clonic convulsion, duration of tonic convulsion
- incidence (number of mice showing convulsions); mortality

1.3.5. Maximal electroshock (MES) induced convulsions:
The mice were randomly divided into groups as described above. The induction of MES induced convulsion was carried out according to previously described method [20].

1.3.6. Electrical Kindling convulsions in rats:
Kindling results from repetitive subconvulsive electrical stimulation of certain areas of the brain. The animals received two subconvulsive electric shocks per day of 21 mA for 0.1 sec, 3 h apart using auricular electrodes, until all animals exhibited grade 4–5 seizure score according to previously described method [21]. The rats were randomly divided into groups as described above. Kindling stimulus was given 45 min after intraperitoneal (i.p.) administration of vehicle (dispensing alcohol), distilled water or test drug (*Argentum Metallicum*) and 30 min after the standard (diazepam) drug. Convulsion scores of *Argentum Metallicum* treated rats were compared with vehicle and diazepam treated animals.

1.4. Biochemical evaluation:
1.4.1. Brain GABA estimation:
Brain GABA level was estimated according to previously described method [22] using GABA as a standard.

1.4.2. Estimation of total protein:
Protein concentration was estimated according to previously described method [23] using BSA (bovine serum albumin) as a standard.

1.4.3. Estimation of nitrite/nitrate level:
The NO level was estimated as nitrite by the acidic Griess reaction after reduction of nitrate to nitrite by vanadium trichloride according to previously described method [24].

1.4.4. Estimation of Xanthine oxidase (XO) level:
Xanthine oxidase activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbency at 293 nm, according to previously described method [25].

1.5. Statistical analysis:

Data were expressed as mean ± standard error mean (SEM). The data of 'Brain GABA', 'Nitric oxide' and 'Xanthine oxidase' was analyzed using one-way analysis of variance (ANOVA), Dunnett's multiple range test was applied for post hoc analysis. Data of 'incidence of convulsion' was analyzed by Chi^2 test. Data of 'mortality' was analyzed by Fisher's exact test. Data of 'percentage seizure free rats' was analyzed by using Kaplan–Meier analysis, Log-rank (Mantel-Cox) test was applied for post hoc analysis. Analysis of all the statistical data was performed using GraphPad Prism 5.0 (GraphPad, San Diego, USA). $P < 0.05$ was considered as statistically significant.

RESULTS

3.1. Effects of Argentum Metallicum and diazepam on pentylenetetrazole induced convulsions in mice:

Intraperitoneal administration of PTZ (90 mg/kg) caused hind-limb, tonic-clonic convulsion as well as lethality in mice. Mice pretreated with AM (200 CH and 1 M) significantly and dose dependently protect from PTZ induced convulsion. It significantly ($P < 0.01$ and $P < 0.001$ respectively) delayed the onset of convulsion as compared to PTZ control mice. Similarly when compared to PTZ control mice, AM (200 CH and 1 M) pretreated mice showed significant and dose dependant ($P < 0.01$ and $P < 0.001$ respectively) reduction in duration of tonic convulsion but it failed to produce any significant reduction in duration of clonic convulsion (Fig. 1). Pretreatment with AM (200 CH and 1 M) also significantly reduced ($P < 0.01$ and $P < 0.001$ respectively) the total number of animals convulsed per group along with the percentage mortality as compared to PTZ control mice. Distilled water (DW) (0.4 ml, i.p.) did not show any significant protection against incident of convulsion but it significantly decreased ($P < 0.01$) PTZ induced mortality in the mice (Table 1).

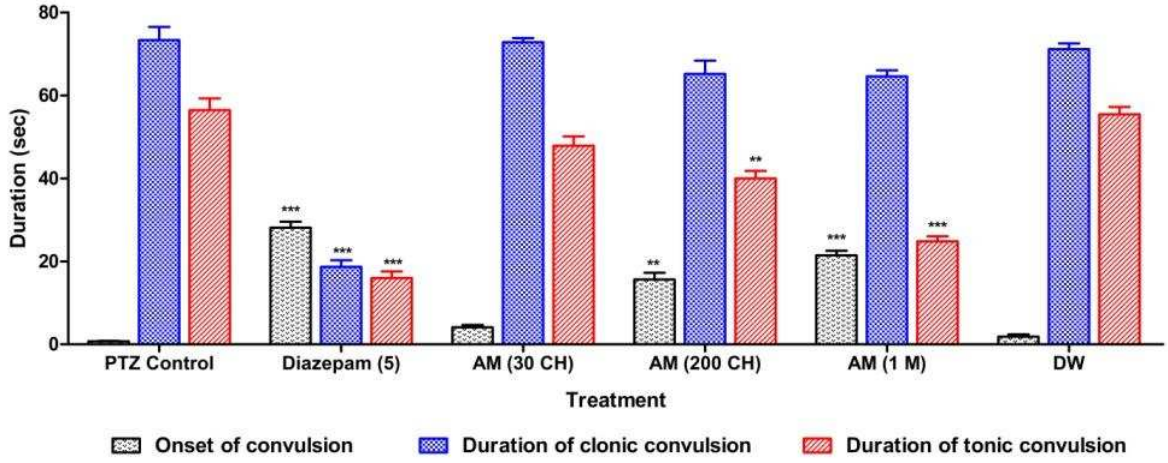

Fig 1. Effects of *Argentum Metallicum* and diazepam on pentylenetetrazole induced convulsions in mice

*Data are expressed as mean ±S.E.M. n = 6 in each g roup. Data was analyzed by one-way ANOVA followed by Dunnett's test (*P < 0.05, **P < 0.01 and ***P < 0.001 as compared to PTZ treated mice.*

Mice treated with PTZ (90 mg/kg) showed significant decreased in brain GABA level ($P < 0.001$) whereas the level of NO and XO were significantly increased ($P < 0.001$) as compared to normal mice (Table 1). Pretreatment with AM (200 CH and 1 M) results significant and dose dependant ($P < 0.01$ and $P < 0.001$ respectively) increased in brain GABA level as compared to PTZ control mice. On other hand it significantly attenuated ($P < 0.05$ and $P < 0.001$ respectively) this elevated level of NO and XO as compared to PTZ control mice. DW (0.4 ml, i.p.) failed to produced any significant change in the level of brain GABA, NO and XO. When compared with PTZ control mice, diazepam (5 mg/kg, i.p.) treated mice significantly antagonized ($P < 0.001$) this changes in brain GABA, NO and XO levels (Table 1).

Table 1. Effects of *Argentum Metallicum* and diazepam on pentylenetetrazole induced convulsions in mice

Treatment				No. convulsed / No. used	% animals protected	Mortality (% Death)	Brain GABA (ng/gm)	Nitric oxide (µmole/g of protein)	Xanthine oxidase (U/g of protein)
PTZ (mg/kg, i.p.)	DZP (mg/kg, i.p.)	AM (0.4 ml, i.p.)	DW (ml, i.p.)						
--	--	--	--	--	--	--	51.70 ± 1.89	0.12 ± 0.012	3.16 ± 0.22
90	--	--	--	6/6	0.00	5/6 (83.33)	27.86 ± 2.03$^{###}$	0.24 ± 0.007$^{###}$	7.14 ± 0.12$^{###}$
90	5	--	--	0/6$^{@@@}$	100.00	0/6 (0.00)$^{$$$}$	46.08 ± 2.55***	0.14 ± 0.010***	3.96 ± 0.20***
90	--	30 CH	--	5/6	16.67	3/6 (50.00)$^{$$}$	28.98 ± 2.48	0.23 ± 0.007	6.80 ± 0.11
90	--	200 CH	--	3/6$^{@@}$	50.00	1/6 (16.67)$^{$$$}$	36.90 ± 1.94**	0.20 ± 0.014*	5.90 ± 0.39*
90	--	1 M	--	1/6$^{@@@}$	83.33	0/6 (0.00)$^{$$$}$	44.82 ± 1.56***	0.15 ± 0.009***	4.58 ± 0.15***
90	--	--	0.4	6/6	0.00	3/6 (50.00)$^{$$}$	28.56 ± 1.12	0.23 ± 0.007	6.66 ± 0.45

*Data are expressed as mean ±S.E.M. n = 6 in each group. Data of 'Brain GABA', 'Nitric oxide' and 'Xanthine oxidase' was analyzed by one-way ANOVA followed by Dunnett's test (*P < 0.05, **P < 0.01 and ***P < 0.001 as compared to PTZ treated mice where as $^{#}$P < 0.05, $^{##}$P < 0.01 and $^{###}$P < 0.001 as compared to normal mice. Data of 'incidence of convulsion' was analyzed by Chi^{2} test ($^{@}$P < 0.05, $^{@@}$P < 0.01 and $^{@@@}$P < 0.001 as compared to PTZ treated mice). Data of 'mortality' was analyzed by Fisher's exact test ($^{$}$P < 0.05, $^{$$}$P < 0.01, $^{$$$}$P < 0.001 as compared to PTZ treated mice).*

3.2. Effects of Argentum Metallicum and diazepam on picrotoxin induced convulsions in mice:

Mice treated with picrotoxin (3.5 mg/kg s.c.) showed clonic convulsions followed by THLE (after some clonic episodes) and mortality in mice. It also resulted in significant decreased ($P < 0.001$) in level of brain GABA and significant increased ($P < 0.001$) in NO and XO level as compared to normal mice. Mice treated with AM (30 CH, 200 CH and 1 M) failed to produced any delayed in onset of convulsion as well as decreased in duration of clonic convulsion. AM (1 M) treated mice significantly decreased duration of tonic convulsion ($P < 0.05$) as compared to PTX control mice whereas mice treated with AM (30 CH, 200 CH) did not produced any significant reduction in duration of tonic convulsion (Fig. 2). Pretreatment with AM (30 CH, 200 CH) as well as DW (0.4 ml, i.p.) failed to show any significant protection against incidence of convulsions and PTX-induced mortality. But mice pretreated with AM (1 M) showed significant protection ($P < 0.05$) against PTX-induced mortality (Table 2).

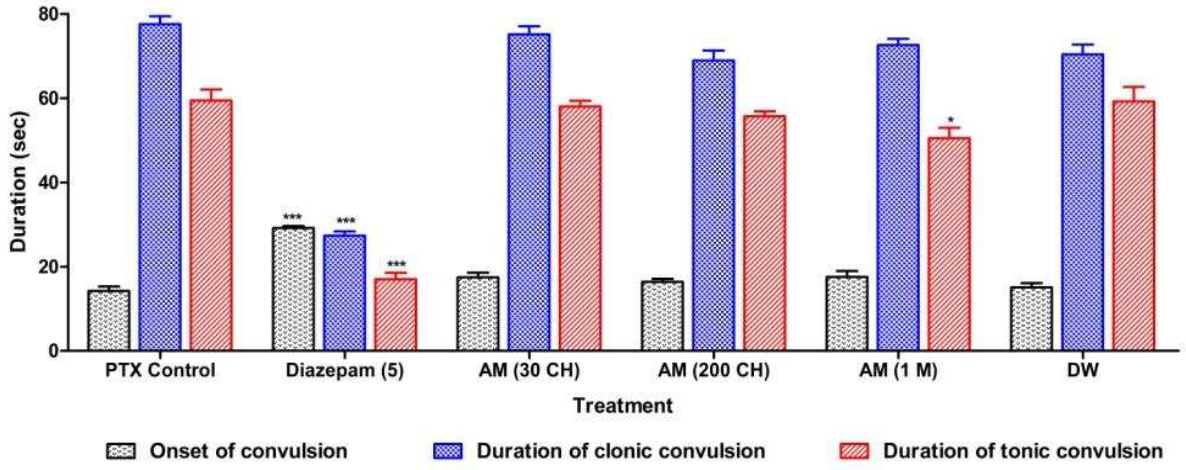

Fig 2. Effects of *Argentum Metallicum* and diazepam on picrotoxin induced convulsions in mice

*Data are expressed as mean ±S.E.M. n = 6 in each group. Data was analyzed by one-way ANOVA followed by Dunnett's test (*P < 0.05, **P < 0.01 and ***P < 0.001 as compared to PTX treated mice.*

As shown in table 2, pretreatment with AM (30 CH, 200 CH and 1 M) and DW (0.4 ml, i.p.) did not produce any significant increased in brain GABA level as well as significant reduction in brain NO and XO level as compared to

PTX control mice. Mice pretreated with diazepam (5 mg/kg, i.p.) significantly antagonized ($P < 0.001$) this elevated level of brain NO and XO whereas it also significantly attenuated ($P < 0.001$) this decreased level of brain GABA.

Table 2. Effects of *Argentum Metallicum* and diazepam on picrotoxin induced convulsions in mice

Treatment				No. convulsed / No. used	% animals protected	Mortality (% Death)	Brain GABA (ng/gm)	Nitric oxide (µmole/g of protein)	Xanthine oxidase (U/g of protein)
PTX (mg/kg, s.c.)	DZP (mg/kg, i.p.)	AM (0.4 ml, i.p.)	DW (ml, i.p.)						
--	--	--	--	--	--	--	52.25 ± 0.74	0.11 ± 0.010	3.30 ± 0.23
3.5	--	--	--	6/6	0.00	5/6 (83.33)	23.22 ± 1.28$^{###}$	0.24 ± 0.005$^{###}$	7.10 ± 0.12$^{###}$
3.5	5	--	--	0/6$^{@@@}$	100.00	0/6 (0.00)$^{\$\$\$}$	49.58 ± 1.96***	0.14 ± 0.007***	3.78 ± 0.17***
3.5	--	30 CH	--	6/6	0.00	5/6 (83.33)	24.14 ± 1.09	0.23 ± 0.007	6.40 ± 0.17
3.5	--	200 CH	--	6/6	0.00	5/6 (83.33)	25.98 ± 0.80	0.21 ± 0.008	6.74 ± 0.16
3.5	--	1 M	--	5/6	16.67	4/6 (66.67)$^{\$}$	23.62 ± 1.25	0.21 ± 0.004	6.62 ± 0.13
3.5	--		0.4	6/6	0.00	5/6 (83.33)	24.14 ± 1.10	0.22 ± 0.009	6.93 ± 0.12

*Data are expressed as mean ± S.E.M. n = 6 in each group. Data of 'Brain GABA', 'Nitric oxide' and 'Xanthine oxidase' was analyzed by one-way ANOVA followed by Dunnett's test (*P < 0.05, **P < 0.01 and ***P < 0.001 as compared to PTX treated mice where as $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ as compared to normal mice. Data of 'incidence of convulsion' was analyzed by Chi^2 test ($^{@}P < 0.05$, $^{@@}P < 0.01$ and $^{@@@}P < 0.001$ as compared to PTX treated mice). Data of 'mortality' was analyzed by Fisher's exact test ($^{\$}P < 0.05$, $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$ as compared to PTX treated mice).*

3.3. Effects of Argentum Metallicum and phenytoin on strychnine induced convulsions in mice:

The production of clonic convulsions followed by THLE and mortality in mice was observed after intraperitoneal administration of strychnine (5.0 mg/kg). When compared with normal mice a significant increased ($P < 0.001$) in brain NO and XO was observed after single dose of strychnine. Treatment with AM (30 CH, 200 CH and 1 M) and DW (0.4 ml, i.p.) did not show any delayed in onset of convulsion as well as decreased in duration of clonic convulsion. It also failed to produce any significant decrease in elevated level of XO and NO as compared to STR treated mice. Mice treated with AM (200 CH and 1 M) significantly antagonized STR induced mortality in mice (Fig. 3 and Table 3).

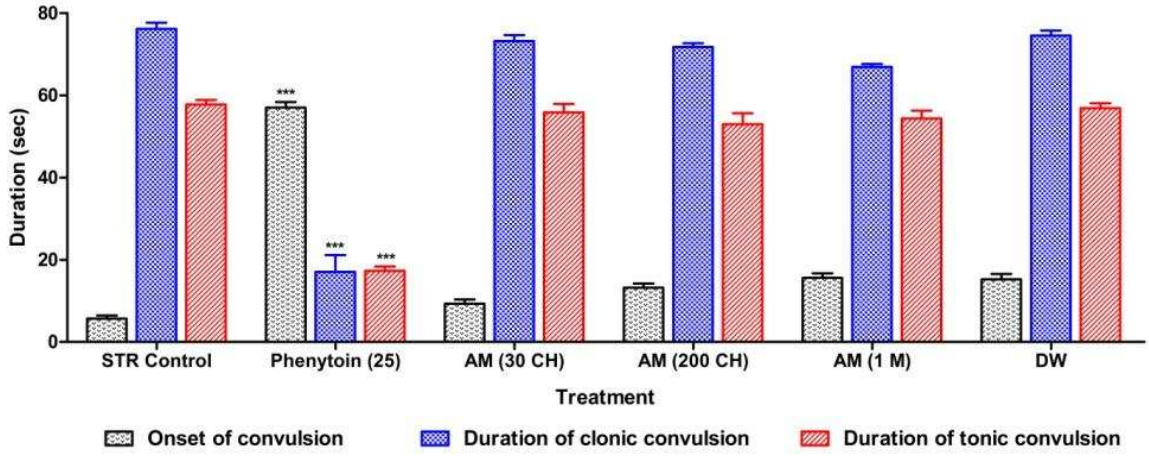

Fig 3. Effects of *Argentum Metallicum* and phenytoin on strychnine induced convulsions in mice

*Data are expressed as mean ± S.E.M. n = 6 in each group. Data was analyzed by one-way ANOVA followed by Dunnett's test (*P < 0.05, **P < 0.01 and ***P < 0.001 as compared to STR treated mice.*

As depicted in Fig. 3 mice treated with phenytoin (25 mg/kg) significantly delayed ($P < 0.001$) onset of convulsion and decreased duration clonic-tonic convulsion as compared to STR control mice. When compared with STR

control mice, the elevated level of NO and XO in brain was significantly attenuated ($P < 0.001$) by treatment with phenytoin (25 mg/kg). It also showed significant protection ($P < 0.05$) against STR-induced mortality (Table 3).

Table 3. Effects of *Argentum Metallicum* and phenytoin on strychnine induced convulsions in mice

Treatment				No. convulsed / No. used	% animals protected	Mortality (% Death)	Nitric oxide (µmole/g of protein)	Xanthine oxidase (U/g of protein)
STR (mg/kg, i.p.)	PHY (mg/kg, i.p.)	AM (0.4 ml, i.p.)	DW (ml, i.p.)					
--	--	--	--	--	--	--	0.12 ± 0.009	3.10 ± 0.24
5	--	--	--	6/6	0.00	5/6 (83.33)	0.23 ± 0.007$^{###}$	7.40 ± 0.21$^{###}$
5	25	--	--	0/6$^{@@@}$	100.00	0/6 (0.00)$^{$$$}$	0.14 ± 0.010***	4.60 ± 0.28***
5	--	30 CH	--	6/6	0.00	5/6 (83.33)	0.23 ± 0.015	6.90 ± 0.07
5	--	200 CH	--	6/6	0.00	4/6 (66.67)$^{$}$	0.22 ± 0.013	6.54 ± 0.16
5	--	1 M	--	6/6	0.00	4/6 (66.67)$^{$}$	0.21 ± 0.006	6.82 ± 0.16
5	--	--	0.4	6/6	0.00	5/6 (83.33)	0.22 ±0.009	6.62 ± 0.23

*Data are expressed as mean ±S.E.M. n = 6 in each group. Data of 'Nitric oxide' and 'Xanthine oxidase' was analyzed by one-way ANOVA followed by Dunnett's test (*P < 0.05, **P < 0.01 and ***P < 0.001 as compared to STR treated mice where as $^{#}P < 0.05$, $^{##}P < 0.01$ and $^{###}P < 0.001$ as compared to normal mice. Data of 'incidence of convulsion' was analyzed by Chi^2 test ($^{@}P < 0.05$, $^{@@}P < 0.01$ and $^{@@@}P < 0.001$ as compared to STR treated mice). Data of 'mortality' was analyzed by Fisher's exact test ($^{$}P < 0.05$, $^{$$}P < 0.01$, $^{$$$}P < 0.001$ as compared to STR treated mice).*

3.4. Effects of Argentum Metallicum and diazepam on isoniazid induced convulsions in mice:

Isoniazid (300 mg/kg i.p.) elicited tonic-clonic convulsions followed by THLE and mortality in mice. Mice treated with AM (30 CH, 200 CH and 1 M) significantly delayed ($P < 0.05$, $P < 0.05$ and $P < 0.001$ respectively) onset of convulsion as compared to INH control mice. There was significant reduction in the duration of clonic and tonic convulsion ($P < 0.05$ and $P < 0.01$ respectively) in AM (1 M) treated mice as compared to INH treated mice. AM (200 CH) treated mice showed significant reduction ($P < 0.01$) in tonic convulsion as compared to INH treated mice. Mice treated with AM (30 CH) failed to produce any significant reduction in clonic-tonic convulsion as compared to INH control mice (Fig. 4). As depicted in table 4, treatment with AM (200 CH and 1 M) significantly protects ($P < 0.05$ and $P < 0.01$ respectively) against convulsions induced by single dose of INH as compared to INH control mice. Treatment with AM (30 CH, 200 CH and 1 M) also significantly antagonized ($P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively) INH induced mortality in the mice.

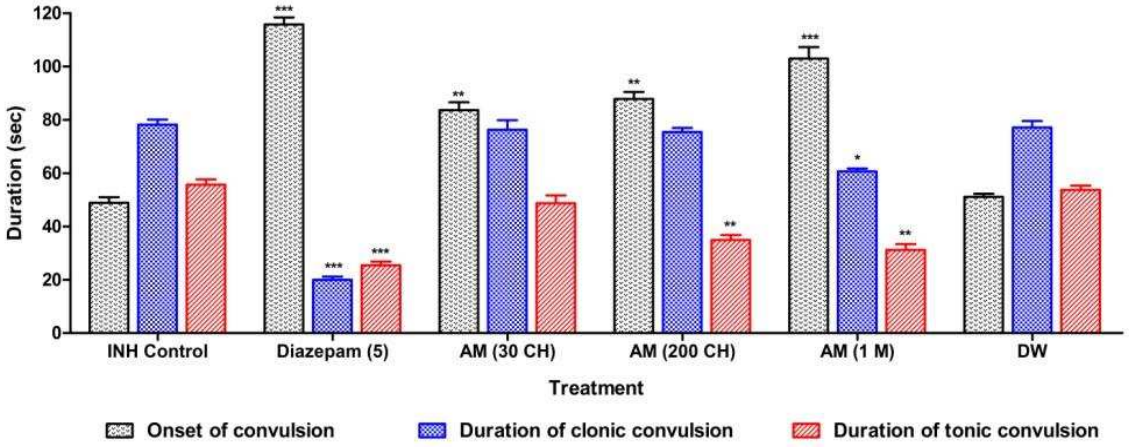

Fig 4. Effects of *Argentum Metallicum* and diazepam on isoniazid induced convulsions in mice

*Data are expressed as mean ±S.E.M. n = 6 in each group. Data was analyzed by one-way ANOVA followed by Dunnett's test (*P < 0.05, **P < 0.01 and ***P < 0.001 as compared to INH treated mice.*

There was significant increased ($P < 0.001$) in the brain XO and NO level as well as significant decreased ($P < 0.001$) in the brain GABA level in INH treated mice as compared to normal mice. Mice treated with AM (200 CH and 1 M) showed significant and dose dependant attenuation ($P < 0.05$, $P < 0.01$ respectively) of this endogenous enzyme levels as compared to INH control mice (Table 2). Mice treated with DW (0.4 ml, i.p.) did not produce any significant protection against INH induced convulsion as compared to INH control mice. However, when compared

with INH control mice, treated with diazepam (5 mg/kg, i.p.) significantly attenuated ($P < 0.001$) INH induced convulsion as well as endogenous enzyme levels.

Table 4. Effects of *Argentum Metallicum* and diazepam on isoniazid induced convulsions in mice

Treatment				No. convulsed / No. used	% animals protected	Mortality (% Death)	Brain GABA (ng/gm)	Nitric oxide (µmole/g of protein)	Xanthine oxidase (U/g of protein)
INH (mg/kg, i.p.)	DZP (mg/kg, i.p.)	AM (0.4 ml, i.p.)	DW (ml, i.p.)						
--	--	--	--	--	--	--	50.18 ± 0.79	0.12 ± 0.024	3.58 ± 0.41
300	--	--	--	6/6	0.00	5/6 (83.33)	22.98 ± 1.92$^{###}$	0.21 ± 0.009$^{###}$	6.54 ± 0.44$^{###}$
300	5	--	--	0/6$^{@@@}$	100.00	0/6 (0.00)$^{$$$}$	47.68 ± 1.26***	0.14 ± 0.007***	4.64 ± 0.19***
300	--	30 CH	--	6/6	0.00	4/6 (66.67)$^{$}$	24.46 ± 1.06	0.20 ± 0.018	6.64 ± 0.12
300	--	200 CH	--	4/6$^{@}$	33.33	2/6 (33.33)$^{$$}$	28.96 ± 1.28*	0.19 ± 0.012*	5.62 ± 0.15*
300	--	1 M	--	2/6$^{@@}$	66.67	1/6 (16.67)$^{$$$}$	37.76 ± 1.74**	0.15 ± 0.008**	4.85 ± 0.08**
300	--	--	0.4	6/6	0.00	5/6 (83.33)	22.80 ± 1.00	0.21 ± 0.005	6.80 ± 0.13

*Data are expressed as mean ± S.E.M. n = 6 in each group. Data of 'Brain GABA', 'Nitric oxide' and 'Xanthine oxidase' was analyzed by one-way ANOVA followed by Dunnett's test (*P < 0.05, **P < 0.01 and ***P < 0.001 as compared to INH treated mice where as $^{#}P < 0.05$, $^{##}P < 0.01$ and $^{###}P < 0.001$ as compared to normal mice. Data of 'incidence of convulsion' was analyzed by Chi^2 test ($^{@}P < 0.05$, $^{@@}P < 0.01$ and $^{@@@}P < 0.001$ as compared to INH treated mice). Data of 'mortality' was analyzed by Fisher's exact test ($^{$}P < 0.05$, $^{$$}P < 0.01$, $^{$$$}P < 0.001$ as compared to INH treated mice).*

3.5. Effects of Argentum Metallicum and phenytoin on MES induced convulsions in mice:

Vehicle control mice developed tonic flexion of the limbs followed by tonic extension of hind limbs (THLE) after the MES test. There was significant ($P < 0.05$ and $P < 0.001$) and dose dependant reduction in the duration of THLE in mice treated with AM (200 CH and 1 M) as compared to vehicle control mice. It also significantly and dose dependently ($P < 0.05$ and $P < 0.01$) attenuated incidence of convulsion induced after MES test. Mice treated with AM (30 CH, 200 CH and 1 M) significantly decreased ($P < 0.05$, $P < 0.001$ and $P < 0.001$) the mortality induced after MES test. DW (0.4 ml, i.p.) failed to produce any significant protection against MES induced convulsion. Mice treated with phenytoin (25 mg/kg) significantly decreased ($P < 0.001$) duration of TLHE as well as MES induced mortality and incidence of convulsion in mice as compared to vehicle control mice (Table 5).

Table 5. Effects of *Argentum Metallicum* and phenytoin on MES induced convulsions in mice

Treatment			No. convulsed / No. used	% animals protected	Duration of THLE (Sec) (Mean ± S.E.M.)	Mortality (% Death)
PHY (mg/kg, i.p.)	AM (0.4 ml, i.p.)	DW (ml, i.p.)				
--	--	--	6/6	0.00	15.98 ± 0.88	5/6 (83.33)
25	--	--	0/6$^{@@@}$	100.00	1.54 ± 0.27***	0/6 (0.00)$^{$$$}$
--	30 CH	--	5/6	16.67	14.38 ± 1.19	2/6 (33.33)$^{$$}$
--	200 CH	--	4/6$^{@}$	33.33	11.66 ± 0.79*	1/6 (16.67)$^{$$$}$
--	1 M	--	2/6$^{@@}$	66.67	7.34 ± 0.72***	0/6 (0.00)$^{$$$}$
--	--	0.4	6/6	0.00	15.44 ± 1.32	5/6 (83.33)

*Data are expressed as mean ± S.E.M. n = 6 in each group. Comparison was made with vehicle control group for each test. Data of 'duration' was analyzed by One-way ANOVA followed by Dunnett's test (*P < 0.05, **P < 0.01, ***P < 0.001). Data of 'incidence of convulsion' was analyzed by Chi^2 test ($^{#}P < 0.05$, $^{##}P < 0.01$and $^{###}P < 0.001$). Data of 'mortality' was analyzed by Fisher's exact test ($^{$}P < 0.05$, $^{$$}P < 0.01$, $^{$$$}P < 0.001$).*

3.6. Effects of Argentum Metallicum and phenytoin on Electrical kindling in rats:

Full kindling in the rats was confirmed by THLE phase in 6 days by auricular stimulation twice daily. There was significant reduction in the severity of electrically kindled seizures in rats treated with AM (200 CH and 1 M, $P < 0.01$ and $P < 0.001$ respectively) as compared to vehicle treated rats. However, Rats treated with AM (200 CH) and DW (0.4 ml, i.p.) failed to produced any significant reduction in the severity of electrically kindled seizures when compared with vehicle treated rats. When compared with vehicle treated rats, treatment with phenytoin (25 mg/kg)

significantly attenuated ($P < 0.001$) this severity of electrically kindled seizures (Fig. 5A). In vehicle treated group all the rats were undergoes seizures on the 4th day whereas treatment with AM (200 CH) and phenytoin (25 mg/kg) significantly attenuated ($P < 0.001$) total number of rats seizure up to 6th day (Fig. 5B).

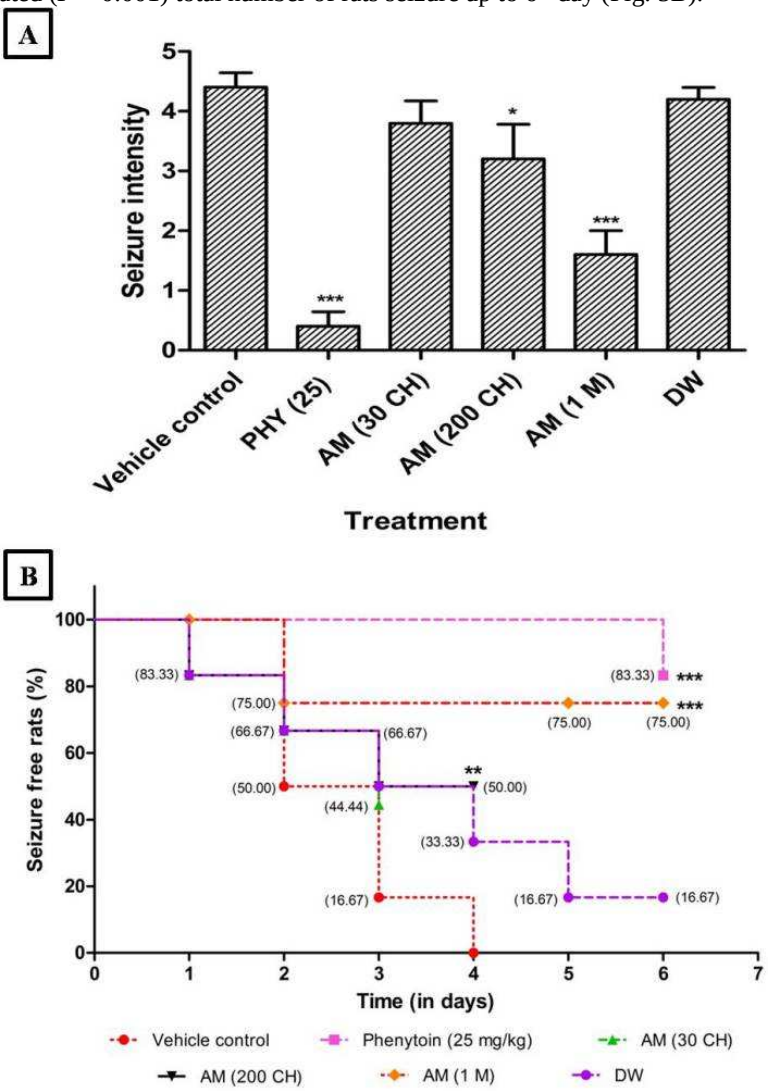

Fig 5. Effects of *Argentum Metallicum* and phenytoin on electrical kindling in rats

(A) Seizure intensity in fully kindled rats

*Data are expressed as mean ± S.E.M. n = 6 in each g roup. Comparison was made with vehicle control group for each test. Data was analyzed by One-way ANOVA followed by Dunnett's test *P < 0.05, **P < 0.01 and ***P < 0.001 as compared to vehicle control rats.*

(B) Kaplan–Meier analysis of the time of spontaneous seizure appearance after sub convulsive electrical stimulation

*The log rank test revealed a significant (*P < 0.05, **P < 0.01, ***P < 0.001) difference between the different group. (n=6)*

DISCUSSION

Epilepsy is exhibited due to an array of underlying pathological phenomenon including kindling, disregulation of sequential firing of neurons, over expression of Na^+ channel, inhibition of glycine synthesis, down regulation of nitroso-oxidative stress leading to flexion and extension of limbs [26]. These therapeutic strategies were traced in our investigation. Moreover, the roles of various metals in neurodegenerative processes have been well documented [27]. Hence present investigation was designed to understand the mechanism and evaluate the therapeutic profile of *Argentum Metallicum* in treatment of experimental epilepsy in animals.

PTZ induces neuropathological aberrations in laboratory animals akin to human. PTZ is a competitive antagonist at GABA mediated Cl^- channel complex. It has been a valid model to screen antiepileptic drugs [28]. DZP is an agonist facilitating opening of Cl^- channel leading to discernable alleviation of seizures [29]. A similar dose effect profile was noticed in the animals treated with *Argentum Metallicum*. The elevated levels of GABA provide evidence to this mechanism.

It has been well documented that oxidative stress plays an important role in the etiology of epilepsy [30, 31]. In case of chemically induced seizures, the presence of oxygen free radicals may be caused by inducing agents themselves. Xanthine oxidase is a major potential source of oxygen free radicals. The burst of XO-mediated free radical generation in the tissue is triggered by a large increase in substrate formation, which occur secondary to degradation of adenine nucleotides [25]. NO acts as a potential proconvulsant [32]. Nitrosative and oxidative stress are mutually synergistic malefic factors precipitating seizures. *Argentum Metallicum* successfully modulated the levels of NO and XO in whole brain homogenate to exhibit antiepileptic activity. The finding of the present investigation is in accordance with the previous study depicting role of *Argentum Metallicum* in regulation of NO level [33].

PTX is GABA receptor antagonist which blocks the Cl^- ion channels link to $GABA_A$ receptor complex which leading to convulsion [34]. Diazepam up regulates GABAergic neurotransmission by increasing chloride ion flux through the chloride-ion channels at $GABA_A$-receptor complex [35]. *Argentum Metallicum* could not significantly ameliorate its effect. The underlying mechanism could be the inability of *Argentum Metallicum* to act on receptor complex directly.

INH induces seizures by interfering with GABA synthesis through inhibition of glutamic acid decarboxylase (GAD) activity, leading to rapid depletion of GABA [36]. *Argentum Metallicum* significantly protects the animals from INH induced seizures in a dose dependant manner and therapeutic effect could be explained by the GABA potentiating profile of AM which might be due to up regulation of GAD activity. Moreover, it has antioxidant and free radical scavenging property which modulates XO level in brain. Our results provide credence to the previous studies carried out by Woo et al [37].

STR causes seizures due to inhibition of glycine synthesis [38]. Diazepam exerts its anticonvulsant property mediated through the glycinergic pathway. *Argentum Metallicum* does not seem to interfere in this cascade as the behavioral symptoms of epilepsy where not ameliorated.

Kindling and MES are conditions where neuronal firing is up regulated due to repetitive administration of exogenous high-frequency electric stimulations leading to uncoordinated and excessive neuronal firing. This is an electrophysiological process independent of GABA level and GABA receptor complex [39]. Neuronal stabilization is a phenomenon which requires potent modulation of electrochemical gradient playing along the neuron. The action potential needs to be synchronized in order to inhibit MES and kindling induced seizures. Oxidative stress and nitrosative insult are independent of the electropathological processes. GABA levels also fall into a similar trend. *Argentum Metallicum* seems to bypass this pathway and exhibit a selective mode of antiepileptic activity to prevent the spread of seizure discharge from the epileptic focus in brain and suppressing generalized tonic-clonic and partial seizures. Hence, the finding of experiment could be explained by citing the effect of *Argentum Metallicum* on selective pathological processes which seems to orchestrate a contraption.

Recently a homeopathic preparation of Belladonna has been screened in dogs for treatment of idiopathic epilepsy which was found to reduce tonic-clonic seizures [14]. Stanton (1981) also reported that the level of test-induced anxiety was significantly reduced by treatment of Argentum in a trial of 40 students and this effect appeared to persist over time [40].

Outcomes form the PTZ model suggest that the *Argentum Metallicum* exerts its anticonvulsant potential through GABAegic mechanism and by modulation of endogenous antioxidant like NO and XO which is further confirmed by results in INH model. It also blocked seizure spread in MES and thus attenuated tonic extension.

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