A Study on Analgesic activity of Matricaria chamomilla

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Abstract

Background: For almost a hundred years homeopaths have attempted to demonstrate the existence of the "potency effect" scientifically. The general implication of all this work is that highly dilute solutions (Homoeopathic potencies) do have effects that can be demonstrated in carefully controlled laboratory experiments. The present study aims to evaluate the effects of high potency in experimental animal and to evaluate the effects of 3x, 30c and 0/3 potencies of *Matricaria Chamomilla* on Central Nervous System.

Methods: The blind screening methodologies, well known in modern Psycho- Neuro pharmacology (Acto- photometer, Rota – rod apparatus, Eddy's Hot plate, Maximal – electro shock method) were utilized for Central Nervous System action investigations.

Results: The results were statistically processed by Analysis of variance test (ANOVA) to compare the effects on treatment groups before and after treatment with control group and Dunnet's *t* test were used to determine the effect of each treatment against control group. The results shows that *Matricaria chamomilla* 0/3, significantly reduce locomotor activity, exhibit muscle relaxant activity, causing analgesia and shows anti-convulsant activity than *Matricaria chamomilla* 3x and *Matricaria chamomilla* 30c.

Conclusion: This experimental study, revealed that high dilution when get dynamised by the process of succussion can have a significant effect on biological system. The central nervous system depression is significantly performed by 0/3 potencies than 3x and 30c.

(Keywords – Matricaria chamomilla, C.N.S stimulant/depressant activity, muscle relaxant activity, Analgesic activity, Anti convulsant activity)

Introduction

The purpose of fundamental research is to describe and possibly to understand the phenomena purported by homoeopathy, using the experimental method. Experimental method is based on the assumption that any hypothesis should be testable, i. e. measurements can be done to prove or disprove it. To do this, we need specific and carefully selected experimental models.

Similar to clinical study, laboratory research is able to show biological activity of homeopathic remedies that cannot be explained as a placebo response. Laboratory research is also capable of

shedding some new light on how the homeopathic remedies may work. This study intended to verify the effect of *Matricaria Chamomilla* in various potencies on central nervous system of experimental animal and it may help to understand the biological phenomenon of the high potencies.

Aims and Objectives

1. To evaluate the effects of high potency in Experimental Animal.
2. To evaluate the effects of 3x, 30c and 0/3 potencies of *Matricaria chamomilla* on Central nervous system.

Materials and Methods

Materials:

Plant: *Matricaria chamomilla*

The Plant, *Matricaria chamomilla* was collected from Ooty under the supervision of Dr.D.Suresh Baburaj, Survey Officer, Medicinal plants survey and collection unit, Central Council for Research in Homoeopathy, Ooty, India. The mother tincture of *Matricaria chamomilla* was extracted as per the directions given in *Homoeopathic Pharmacopoeia of India* (Vol- 5, 1986)[1]. The 3x and 30c potency of *Matricaria chamomilla* were prepared with acqua distillata and 0/3 potency of *Matricaria chamomilla* brought from a reputed firm, which were used in this experiment.

Animals:

Male albino mice (Swiss strain) were procured from Sri Venkateswara Enterprises, Bangalore, India and bred in the animal house of Vinayaka Mission's College of Pharmacy, Salem, India. They were fed on commercial diet and water adlibitum during the experiments. The pellet food containing 22.5% protein, 72.55% carbohydrate, 5% fat and sufficient vitamins and minerals. The cages were placed in well-ventilated place in the laboratory and were provided with 1.5 inches rice-bran bedding which was changed every day. The room temperature was maintained at 25$\pm$ 1^{o}c . The animals were selected randomly. The study was approved by Institutional Animal Ethical Committee of Vinayaka Mission's college of pharmacy, Salem, South India. Five groups (I-V), each comprising of six animals weighing between (20-25g) were selected.

GROUP-I -Control group without any treatment

GROUP-II - Group treated with standard drugs (Dose according to drugs used)

GROUP III - Group treated with *Matricaria chamomilla* 3x,

10 ml/Kg/ p.o, four times/ day for one day.

GROUP IV- Group treated with *Matricaria chamomilla* 30c,

10 ml/Kg/ p.o, two times/ day for three days.

GROUP V- Group treated with *Matricaria chamomilla* 0/3,

10 ml/Kg/ p.o, two times/ day for three days.

The test was made three hours after the latest administration of drug.

Screening Methods for C.N.S Depressant Drugs.

The blind screening methodology, well known in Psyco-Neuro pharmacology was utilized for Central Nervous System actions investigations.

A) Rota rod with compartments – muscle relaxant property[2, 3]:

First the animal is trained to stand on the rotating rod at slow speeds. Once the animal is trained the platform where the animal will fall is lifted slightly upward to restart the counter. After the counter is switched on the animal will keep on balancing on the rotating rod and at one stage it will fall on the platform and at this stage the counter will stop. The time is noted directly in seconds from the counter. The cut off time to animals to fall from the rotating rod at a recommended speed is fixed at (120 Sec) and those animals stay on the rotating rod more than the cutoff time are discarded from the study.

The same experiment is repeated at different speeds of the rod and also after administering suitable drug to the animal. There is a reset button provided by the side of each counter that is used to reset the counter to Zero after each record is taken. The complete setup is designed to work under 230 V 50 Hz.A.C.

The endurance time in seconds before and after the drug administration is recorded from digital counter. Those animals which fall off from the rod after administration of test drug (e.g. Muscle relaxant drugs) show adjustment difficulty to stand on the rotating rod. This indirectly shows the muscle weakness and inco-ordination of the animals due to the relaxation of skeletal muscles. The

drugs with muscle relaxant activity (Central/Peripheral), Sedatives, Anxiolytic drugs (Diazepam type) will show lesser time of standing when compared to pretreatment.

B) Eddy's hot plate – Analgesic activity[4]:

In this method heat is used as a source of pain. Animals are individually placed on a hot plate maintained at constant temperature (55°C) and the reaction of animals, such as paw licking or jump response is taken as the end point. Analgesics increase the reaction – time.

C) Acto- photo meter – C.N.S stimulant/depressant activity[5, 6]:

1. The instrument consists of a cage which is 30 cm long and 30 cm deep. It has wire mesh at the bottom.

2. Six lights and 6 photocells are placed in the outer periphery of the bottom in such a way that a single mouse can block only one beam.

3. Technically its principle is that a photocell is activated when the rays of light failing on photocells are cut off by animals crossing the beam of light.

4. Photocells are connected to an electronic automatic counter device which counts the number of "cutoffs".

D) Maximal electro - shock method (M.E.S) – Anti convulsant activity[7, 8]:

The maximal electro – shock (M.E.S) induced convulsions in animal represent grand mal type of epilepsy. In M.E.S convulsions electric shock is applied through the corneal electrodes. Through optic stimulation cortical excitation is produced. The M.E.S convulsions are divided into five phases such as tonic flexion, tonic extension, clonic convulsion, stupor and recovery or death. Note the time (in seconds) spent by the animal in each phase of convulsions. A substance is known to possess anticonvulsant property if it reduces or abolishes the extensor phase of M.E.S convulsions. This procedure may be used to produce convulsion both in rats and mice. For rat, 150 mA current is applied for 0.2 seconds and for mice 50 mA current is applied for 0.2 seconds.

Statistical Analysis

The results were statistically processed by Analysis of variance test (ANOVA) to compare the effects on treatment groups before and after treatment with control group and Dunnet's *t* test were used to determine the effect of each treatment against control group.

The null hypothesis (H_0) was assumed that there was no difference in effects of treatments between the treatment groups compared with control group and alternate hypothesis (H_1) was assumed there was significant difference in effect of treatment between the treatment groups compared with control group.

Observation

The mice of untreated, control group showed restless, hyper motility and fast climbing behaviour.

The mice treated with standard drug group did not show greed for eating and drinking. They drank very small quantity of water during the experiment. They showed a decrease in locomotor activity and quietness.

The mice treated with *Matricaria Chamomilla* 3x showed hypomotility, increased threshold to pain stimuli, decreased muscle grip and decreased extensor phase in convulsions.

The mice treated with *Matricaria Chamomilla* 30c showed reduced locomotor activity, increased threshold to pain stimuli, decreased muscle grip and decreased extensor phase in convulsions.

The mice treated with *Matricaria Chamomilla* 0/3 showed hypomotility, increased threshold to pain stimuli, decreased muscle grip and decreased extensor phase in convulsions than group treated with *Matricaria Chamomilla* 3x and *Matricaria Chamomilla* 30c.

Result

Locomotor activity

Before treatment: From table 2, calculated F- value (1.92) is compared with table F- value (4.18) with 4, 25 df at 1% level of significance. Therefore the calculated F value is less than table F value. So the null hypothesis is accepted and there is no significant difference in the locomotor activity among the groups before treatment.

After treatment: From table 3, calculated F- value (5.32) is compared with table F- value (4.18) with 4, 25 df at 1% level of significance. Therefore the calculated F value is greater than table F value. So the null hypothesis is rejected and there is significant difference in the locomotor activity among the groups after treatment.

The standard drug, Phenobarbitone sodium (40mg/Kg, i.p) reduce the locomotor activity by 65.39% (P<0.001), Chamomilla 0/3 (10 ml/Kg, o.p) reduce the locomotor activity by 46.54% (P<0.001), Chamomilla 30c (10 ml/Kg, o.p) reduce the locomotor activity by 26.08% (P<0.001) and Chamomilla 3x (10 ml/Kg, o.p) reduce the locomotor activity by 18.25% (P<0.001).

Muscle relaxant activity:

Before treatment:

From the table 6, calculated F- value (1.09) is compared with table F- value (4.18) with 4, 25 df at 1% level of significance. Therefore the calculated F value is less than table F value. So the null hypothesis is accepted and there is no significant difference in the Muscle relaxant activity among the groups before treatment.

After treatment:

From the table 7, calculated F- value (7.09) is compared with table F- value (4.18) with 4, 25 df at 1% level of significance. Therefore the calculated F value is greater than table F value. So the null hypothesis is rejected and there is significant difference in the Muscle relaxant activity among the groups after treatment.

The standard drug, Diazepam (4mg/Kg, i.p) reduce the fall off time by 49.65% (P<0.001), Chamomilla 3x (10 ml/Kg, o.p) reduce the fall off time by 35.93% (P<0.001), Chamomilla 0/3 (10 ml/Kg, o.p) reduce the fall off time by 16.53% (P<0.05) and Chamomilla 30c (10 ml/Kg, o.p) reduce the fall off time by 9.84% (P<0.05).

Analgesic activity:

Before treatment: From the table 10, calculated F- value (2.72) is compared with table F- value (4.18) with 4, 25 df at 1% level of significance. Therefore the calculated F value is less than table F value. So

the null hypothesis is accepted and there is no significant difference in the Analgesic activity among the groups before treatment.

After treatment:From the table 11, calculated F- value (18.55) is compared with table F- value (4.18) with 4, 25 df at 1% level of significance. Therefore the calculated F value is greater than table F value. So the null hypothesis is rejected and there is significant difference in the Analgesic activity among the groups after treatment.

The standard drug, Pentazocine (5mg/Kg, i.p) increase the reaction time by 65% (P<0.001), Chamomilla 0/3 (10 ml/Kg, o.p) increase the reaction time by 49.03% (P<0.001), Chamomilla 3x (10 ml/Kg, o.p) increase the reaction time by 46.51% (P<0.001) and Chamomilla 30c (10 ml/Kg, o.p) increase the reaction time by 26.98% (P<0.001).

Anti- convulsant activity:

After treatment:

From the table 14, calculated F- value (26.52) is compared with table F- value (4.18) with 4, 25 df at 1% level of significance. Therefore the calculated F value is greater than table F value. So the null hypothesis is rejected and there is significant difference in the Anti-Convulsant activity among the groups after treatment.

The standard drug, Phenytoin (25mg/Kg, i.p) decrease extensor phase to 1.5 seconds (P<0.001), Chamomilla 0/3 (10 ml/Kg, o.p) decrease extensor phase to 4.3 seconds (P<0.001), Chamomilla 3x (10 ml/Kg, o.p) decrease extensor phase to 6.5 seconds (P<0.001) and Chamomilla 30c (10 ml/Kg, o.p) decrease extensor phase to 6.83 seconds (P<0.001).

Discussion

In homoeopathy, *Matricaria chamomilla* is used for unbearable colic and pains in different locations accompanied by an excitation of central nervous system. It is also used in convulsions consecutive to a state of furious agitation. In short, it can be used to depress central nervous system activities[9].
The results of the present study show some inhibitory effects of Chamomilla potencies on central nervous system. The central nervous system depression is significantly performed by 0/3 potencies than 3x and 30c.

Referring to the previous studies in this area, an experimental pharmacological research performed by Guillemain (1983) showed that, at 3c and 4c dilutions, the Chamomilla significantly diminishes the number of fights in rats after exposing to electric shock. The study also showed that Chamomilla 3c and 4c decreased caffeine induced motor hyperactivity in male rats[10]. Another study conducted by Criestea

(1996) Chamomilla 5c has a stimulatory action on central nervous system of experimental animal with hypomotility. This study also reveals that Chamomilla 30c has an inhibitory effect on central nervous system activities of experimental animal with hypermotility[11]. But the results of the present study concurrent with the statement of Richard Hughes that Chamomilla is one of those drugs whose effect of crude and infinitesimal doses are about identical[12]

Table – 13

Anti-convulsant activity against Maximal Electro-shock-induced Convulsions

Group	Treatment	Dose	Flexion (sec)	Extensor (sec)	Clonus (sec)	Recovery/ Death
1	Control	10ml/kg p.o	2.3±0.12	9.6±0.8	1.6±0.21	136±3.98
2	Phenytoin	25mg/kg i.p	0.83±0.06	1.5±0.22	1.16±0.29	17.5±1.43
3	Chamomilla3x	10ml/kg p.o	2±0.25	6.5±0.72	1.5±0.22	142.5±6.64
4	Chamomilla30c	10ml/kg p.o	1.5±0.34	6.83±0.6	1.66±0.3	142.16±7.46
5	Chamomilla0/3	10ml/kg p.o	2±0.25	4.3±0.87	1.5±0.22	132.66±10.17

Number of animals used in each group: 6

The results are in Mean±SEM

Table - 2

Analysis of variance table for locomotor activity before treatment.

Source of variation	df	Sum of squares	Mean sum of squares	F-ratio	F-table value
Treatment	5-1=4	9159.20	2289.8	1.92***	4.18
Error	29-4=25	29712.17	1188.48		
Total	30-1=29	38871.37			

***P > 0.01, df 4, 25.

Table – 3

Analysis of variance table for locomotor activity after treatment.

Source of variation	df	Sum of squares	Mean sum of squares	F-ratio	F-table value
Treatment	5-1=4	26276.13	6569.03	5.32***	4.18
Error	29-4=25	30860.67	1234.42		
Total	30-1=29	38871.37			

***P < 0.01, df 4, 25.

Table - 5 Muscle relaxant property-Rota-rod apparatus

Group	Treatment	Dose	Fall off time in sec before treatment	Fall off time in sec after treatment	% decrease in time
1	Control	10ml/kg p.o	345.83±18.79	349.16±22.08	0.96%
2	Diazepam	4mg/kg i.p	312.5±10.68	157.33±13.11	49..65%
3	Chamomilla3x	10ml/kg p.o	372±31.86	238.33±51.47	35.93%
4	Chamomilla 30c	10ml/kg p.o	335.16±22.60	302.16±19.10	9.84%
5	Chamomilla0/3	10ml/kg p.o	369±28.67	308±17.62	16.53%

Number of animals used in each group: 6

The results are in Mean±SEM

Table - 6

Analysis of variance table for Muscle relaxant activity before treatment.

Source of variation	df	Sum of squares	Mean sum of squares	F-ratio	F-table value
Treatment	5-1=4	14643.53	3660.88	1.09***	4.18
Error	29-4=25	83825.17	3353.01		
Total	30-1=29	98468.7			

***P > 0.01, df 4, 25.

Table – 7

Analysis of variance table for Muscle relaxant activity after treatment.

Source of variation	df	Sum of squares	Mean sum of squares	F-ratio	F-table value
Treatment	5-1=4	134625.66	33656.42	7.09***	4.18
Error	29-4=25	118640.34	4745.61		
Total	30-1=29	253266			

***P < 0.01, df 4, 25.

Table - 8

Dunnet's *t* test table against control for Muscle relaxant activity

Statistic	Diazepam	Chamomilla 3x	Chamomilla 30c	Chamomilla 0/3
t	10.78****	6.23****	2.64**	2.31**

****P < 0.001, **P < 0.05, df 25

Table – 9

Analgesic effect – Eddy's hot plate

Group	Treatment	Dose	Basal reaction time. (sec)	Reaction time after treatment.(sec)	% increase in reaction time
1	Control	10ml/kg p.o	4.66±0.33	4.5±0.22	3.55%
2	Pentazocine	5mg/kg i.p	3.5±0.22	10±0.73	65%
3	Chamomilla3x	10ml/kg p.o	4.3±0.21	6.3±0.44	46.51%

4	Chamomilla30c	10ml/kg p.o	4.6±0.33	6.3±0.42	26.98%
5	Chamomilla0/3	10ml/kg p.o	4.5±0.34	8.83±0.47	49.03%

Number of animals used in each group: 6

The results are in Mean±SEM

Table - 10

Analysis of variance table for Analgesic activity before treatment.

Source of variation	df	Sum of squares	Mean sum of squares	F-ratio	F-table value
Treatment	5-1=4	5.67	1.417	2.72***	4.18
Error	29-4=25	13	0.52		
Total	30-1=29	18.27			

***P > 0.01, df 4, 25.

Table - 11

Analysis of variance table for Analgesic activity after treatment.

Source of variation	df	Sum of squares	Mean sum of squares	F-ratio	F-table value
Treatment	5-1=4	115.8	28.95	18.55***	4.18
Error	29-4=25	39	1.56		
Total	30-1=29	154.8			

***P < 0.01, df 4, 25.

Table - 12

Dunnet's *t* test table against control for Analgesic activity

Statistic	Pentazocine	Chamomilla 3x	Chamomilla 30c	Chamomilla 0/3
t	17.08****	5.59****	5.59****	13.44****

****P < 0.001, df 25.

Table - 14

Analysis of variance table for Anti-Convulsant activity after treatment.

Source of variation	df	Sum of squares	Mean sum of squares	F-ratio	F-table value
Treatment	5-1=4	222.87	55.71	26.52***	4.18
Error	29-4=25	52.5	2.1		
Total	30-1=29	275.37			

***P < 0.01, df 4, 25.

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Dunnet's *t* test table against control for Anti-Convulsant activity

Statistic	Phenytoin	Chamomilla 3x	Chamomilla 30c	Chamomilla 0/3
t	4.53****	1.73**	1.58*	2.96***

****P < 0.001, *** P < 0.01, ** P < 0.1, * P < 0.5, df 25.

Conclusion

In conclusion, the homoeopathic remedy, characterized by infinitesimal doses and by dynamization according to Hahnemann's preparation technique has a marked informational character. The possibility of storing information from molecules can be used for therapeutical as well as experimental purpose.

The result of the study justify the opportunity of future researches concerning the intimate mechanism of inhibitory action on central nervous system of Chamomilla homoeopathic potencies in accordance with principle of similia.

References

1. Chamomilla, *Homoeopathic Pharmacopoeia of India*, Vol- 5, Controller of publications, Government of India, 1986, pp 22.
2. D'Amour, F.E and Smith, D.L; *J. Pharm.Exptl. Therap*. 1941, 72: 74
3. Kulkarni,S.K; *Life Sciences*, 1980, 27 : 185 – 188
4. Eddy, N.B. and Leimbach, D. J; *J. Pharmacol. Exptl. Therap*. 1953, 107: 385
5. Dewis, P.B; *Brit. J. Pharmac. Chemotherap*.1953, 8 : 46
6. Kulkarni, S.K and Dandiya, P.C; *Indian J. Med. Res*.1975, 63: 462-468
7. Misra, A.K; Dandiya, P.C and Kulkarni, S.K; *Indian J. Pharmac*. 1973, 5 : 449-450
8. Kulkarni, S.K; *Arch. Int. Pharmacodyn*. 1981, 252 : 124-132
9. Hahnemann. S. '*Materia Medica Pura*' Vol-1, reprint edition 1992, B.Jain publishers Pvt.Ltd, pp.379-381
10. Guillemain, J. (1983) La recherché pharmacologique appliqué a l'Homeopathie, sa grandeur, ses faiblesses, *Homeop.Fr* 71, pp.191 – 200.
11. Cristea A., Teodorescu – Negres.S, Darie.V *et al* " Chamomilla Homoeopathic Dilution effect on Central Nervous System – An Experimental pharmacological study", *Europ. Neuropsychopharmacol*.3, pp. 446 – 447.
12. Richard Hughes '*A manual of pharmaco - dynamics*' 6 th reprint edition 2001, B.Jain publishers Pvt.Ltd, pp. 382

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