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COLLABORATIVE RESEARCH

# Preliminary studies on analgesic and behavioural activities of the homoeopathic formulations of *Chenopodium ambrosioides* in experimental animal models

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**ABSTRACT:**

*Chenopodium ambrosioides* Linn. (Family: Chenopodiaceae) is traditionally used in the treatment of dysmenorrhoea, uterine haemorrhage and fibroids. In homoeopathy, *C.ambrosioides* is prescribed for worm infestation but its use in providing relief to the subjects suffering from central nervous system disorders is lacking though C*. ambrosioides* has been reported to possess anti - inflammatory and analgesic effect. In the present preliminary study, homoeopathic formulations (3x, 6x, 12x and 30C potencies) of *C.ambrosioides* administered at a dose of 0.5 ml/rat/day were tested for their analgesic (hot plate, ice plate and Randall -Selitto tests) and behavioural (rota rod and open field tests) activities 30 min after administration of drug on 10$^{th}$, 20$^{th}$ and 30$^{th}$ day of the study. The results revealed that all the four potencies of *C.ambrosioides* had increased the latency time required to raise and to lick the fore or hind paw for thermal sensation on hot plate test and for cold sensation on Ice plate test. They had also increased the quantum of threshold pressure to mechanical induced pain on Randall -Selitto test but depressed the motor coordination and locomotor activity. Based on the findings of this study, we report that the homoeopathic formulations of *C.ambrosioides* may possess cns depressant property. However, further detailed studies are needed for a definitive conclusion.

**Keywords:** homoeopathic formulation; *C. ambrosioides* in different potencies; analgesic activity; behavioural effect and albino rats

## Introduction

Plants with a history of use in traditional medicine constitute an obvious starting point in the search for new therapeutically active drugs. A scientific evaluation of medicinal plants according to their traditional claims could be incorporated into the complementary and alternative medicine system. One such plant is *Chenopodium ambrosioides* Linn. (Family: Chenopodiaceous) which has been used in the present investigation. It is an annual plant that grows to about 1 m in height with short leaves. In traditional medicine, seeds and fruits of *C. ambrosioides* are used as anthelmintic chiefly for ascaris[1] and for intestinal amoebae[2]. Decoction prepared from its leaves and flowers is used as remedy for fibroids and uterine haemorrhage.[3, 4] The essential oil of *C. ambrosioides* has been reported to possess antiulcer[5] and antiprotozoal activities in mice[6]. The flavonoids and terpenoids compounds isolated from the plant have antioxidant and anticancer effects[7]. In homoeopathy *C. ambrosioides* has been prescribed for worm infestation[8].

Although, the methanol extract of its leaves has been reported to possess anti-inflammatory effect against carrageenan - induced paw oedema and cotton pellet induced granuloma and an analgesic effect against the thermal stimuli in rats[9] but the homoeopathic formulations of *C. ambrosioides* has not been investigated for their analgesic and behavioural activities. The present preliminary study was therefore undertaken to have some assessment about these effects at different (3x, 6x, 12x and 30c) potencies of *C. ambrosioides* in experimental animal models.

## Materials and Methods:

### Plant collection

The whole plant of *Chenopodium ambrosioides* Linn. (Family: Chenopodiaceae) was collected in August'2007 from the Nilgiris Hills, Tamil Nadu, India and taxonomically identified/authenticated by the

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### Drugs

Homoeopathic formulations of *C. ambrosioides* in 3x, 6x and 12x potencies were prepared in decimal scale while 30 c was prepared in centesimal scale by M/S. Bahola Laboratories, Puducherry from a single batch of whole plants supplied by Survey of Medicinal Plants and Collection Unit, Udagamandalam, Tamilnadu and sent to us for further experimental study.

### Animals

Healthy albino rats (120-140 g) obtained from M/S. Jagan animal's breeder and supplier, Hyderabad were used for the study. They were housed in polypropylene cages and maintained with 12: 12 hrs, light/dark cycles, and room temperature 22-24 °C. The animals were fed with standard pellet (Hindustan Lever, Kolkata, India) diet and water was given *ad - libitum.* The animals were acclimatized to standard laboratory conditions for 10 days and thereafter they were accustomed to respective test procedures by giving them three test trials at 10 min intervals on each day for three days before subjected to experimental protocols.

### Experimental design

A total of 90 rats were taken and grouped into 5 batches of 18 each which were further divided into 6 sub-batches of 3 each. The different potencies (3x, 6x, 12x and 30c) of *C. ambrosioides* were orally administered daily at a dose of 0.5 ml/rat/day for 30 days. Two groups of parallel control, one receiving equivalent volume of alcohol (91.5%v/v; used as a vehicle for preparation of different potencies of the test drug) and other receiving equivalent volume of normal saline were also run simultaneously. The test potencies of *C. ambrosioides and* alcohol were diluted with distilled water in a ratio of 1:4 so that each rat should not receive total volume of more than 2 ml per day. The response of drug was measured after 30 min of its administration on $10^{th}$, $20^{th}$ and $30^{th}$ day. Readings taken just before administration of the drug/alcohol/normal saline on day 1 of the study were considered as the initial control values for comparison. The experimental protocols were approved by IAEC (Institutional Animal Ethics Committee) of Department of Zoology, Osmania University, Hyderabad (Reg. No. 383/01/a/CPCSEA). All the experiments were conducted in air conditioned room.

### Analgesic activity:

Analgesic activity was studied by (i) Hot plate, (ii) Ice plate and (iii) Randall - Selitto tests.

### Hot plate test

The hot plate latency test was carried out based on the method of Eddy *et al*[10]. 30 min after the administration of drug, alcohol or saline, the rats were gently placed individually on a hot plate maintained at a temperature of 55 ± 2ºC. The time taken (in seconds) by the rats to lick the fore or hind paws was noted which was considered as the reaction time (latency time). Control reaction time of the rats to thermal noxious stimulus was taken on day 1 before the administration of drug, alcohol or normal saline was considered as initial value for comparison. A cut-off time of 15 sec [11] was selected to avoid tissue damage.

### Ice plate test

Rats were gently placed individually on the ice cubes (0 - 4 ºC) filled in a container (20 x 20 x 20 cm) and covered with a plastic cover. The reaction time in seconds to lick the fore or hind paw to cold sensation was noted after 30 min of administration of test drug, alcohol or saline. The reaction time (latency time) of the rats to cold sensation was taken on day 1 before the administration of drug, alcohol or normal saline was considered as initial value for comparison. A cut-off reaction time of 15 sec was chosen in order to avoid physical injury to the animals. Percentage of analgesia was calculated as described in hot plate technique[10].

### Randall - Selitto test

The analgesic activity of drug against mechanical induced pain was carried out as described by Randall - Selitto [12]. After 30 min of drug, alcohol or saline administration, the paw of the right foot of the rat was placed on the rubber base of the apparatus (Randall - Selitto apparatus, Ugo Basile, Italy) and pressure (in ponds; expressed in g) was applied either on $2^{nd} – 3^{rd}$ or $3^{rd} – 4^{th}$ metatarsal region through a pointed tip and increased gradually until vocalization elicited which was considered as threshold pressure to mechanical induced pain. Threshold pressure to mechanical induced pain taken on day 1 before the administration of drug, alcohol or normal saline was considered as initial value for comparison.

### Behavioural activity:

Behavioural activity was studied by (i) Rota - rod and (ii) Open field tests.

### Rota - rod test

Motor coordination, grip strengths of the rats were measured by using the automated rota rod apparatus (Dolphin™ instrument)[13]. The rotor was divided in to

three compartments which allowed three rats to test simultaneously at a time. The rats capable of remaining on the rota rod for 60 seconds or more in three successive trials were selected for the study. 30 min after the administration of drug, alcohol or normal saline, rats were placed on the horizontal rod with the head directed opposite to the direction of rotating rod at a speed of 5rpm and duration of the time (sec) that a rat can exist on the rotating rod was measured as grip strength. The grip strengths of the rats were measured on day 1 just before administration of the drug, alcohol or normal saline were considered as initial value for comparison.

### Open - field test

Open field test was used for recording locomotor activity[14]. The apparatus consisted of a wooden (96x96x6cm) box. The floor of the box was divided in to 36 equal squares which was painted alternatively with block and white colours and illuminated with low intensity diffuse light (40 W) placed at a height of 100 cm. The animals were placed gently in the centre of the apparatus one after another where they were free to walk and to get adapted to the new environment. After completion of their training, the animals were treated with test drug, alcohol or saline and 30 min later the animals were placed individually in the apparatus and the number of squares crossed in 5 min was recorded. The floor of the box was cleaned after every trial.

## Statistical analysis:

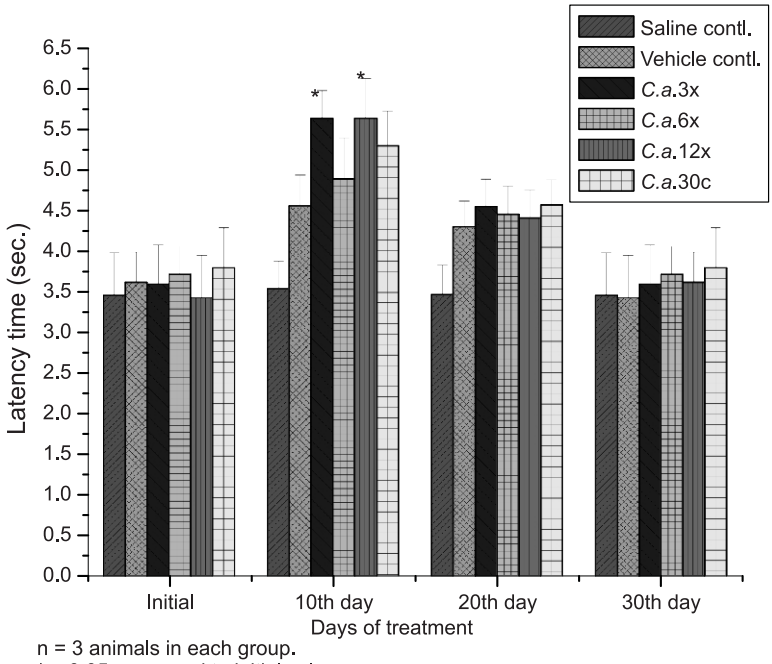
The data were expressed as Mean ± S.E.M. The difference between mean values of groups were statistically analysed by student's 't' test. A difference at *P*- Value < 0.05 was considered to be statistically significant[15].

## Results

### Analgesic activity

### Hot plate test

Fig.1 shows the results of the analgesic effect of different potencies (3x, 6x, 12x and 30c) of *C. ambrosioides* using hot plate assay. The latency time required to raise and lick the hind paw for thermal stimulus was more or less same (3.42 to 3.79 sec) in control (on day 1 before administration of drug/alcohol) and saline treated animals when measured on different days of the experiment. On the other hand, the animals treated with different potencies of *C. ambrosioides* or alcohol at a dose of 0.5 ml/rat/day showed increase (4.55 to 5.63 sec) in the latency time on 10$^{th}$ day when measured after 30 min of drug administration. The difference was significant ($p<0.05$) only with those rats treated with 3x and 12 x potencies. Thereafter, the increase in the duration of latency time tapered off gradually on 20$^{th}$ day and 30$^{th}$ day on continuation of treatment (Fig.1).




n = 3 animals in each group.
*p<0.05 compared to initial value.

Fig.1 Analgesic effect of *C.ambrosioides* (0.5ml/rat/day) on Hot plate test (Mean ± S.E.M.)

### Ice plate test

The results of the analgesic effect of different potencies of *C. ambrosioides* by ice plate assay are shown in Fig.2, There was an increase in the latency time (7.28 to 8.25sec) to cold sensation when measured 30 min after the administration of different potencies (3x, 6x, 12x and 30c) of *C. ambrosioides* or alcohol at a dose of 0.5 ml/rat/day on 10th day. The difference in the increase in latency time to cold sensation was significant ($p<0.05$) with those animals which were treated with 3x and 6x potencies. Thereafter, the increase in the duration of latency time to cold sensation was tapered off gradually on 20th day and 30th day on continuation of the treatment. The initial latency time to cold sensation recorded on day 1 before administration of drug, alcohol or normal saline and 30 min after the administration of normal saline on different days of experimentation was more or less same (5.76 to 6.09 sec) (Fig.2).

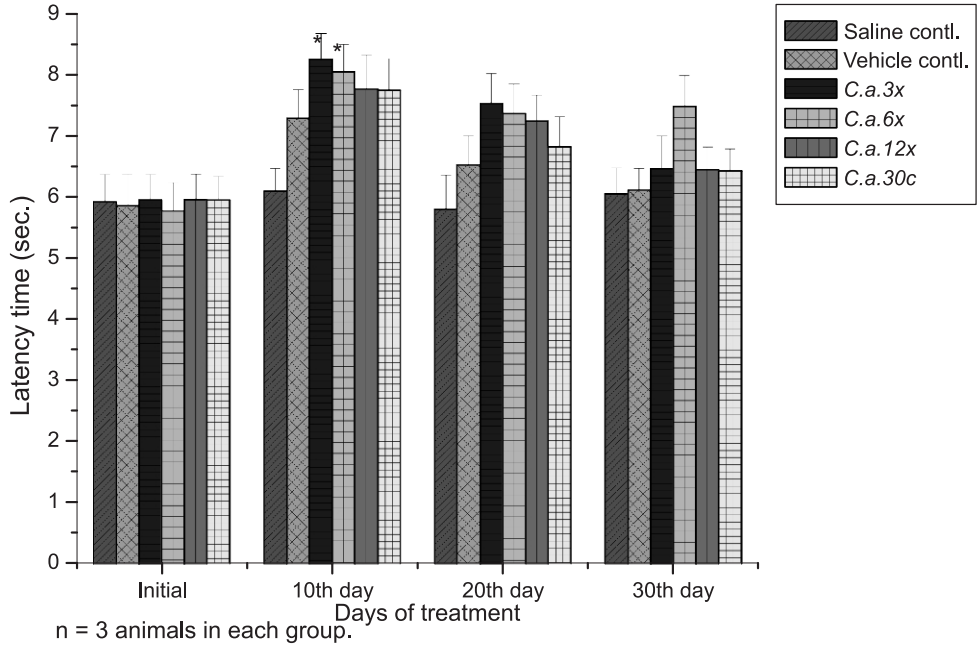
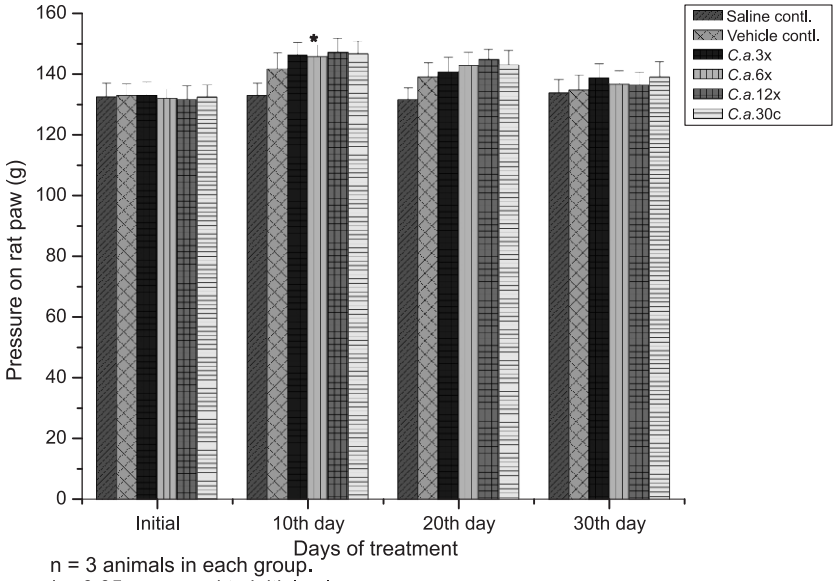



Fig.2 Analgesic effect of *C.ambrosioides* (0.5ml/rat/day) on Ice plate test (Mean ± S.E.M.)

### Randall - Selitto test

Fig. 3 shows the results of the analgesic effect of different potencies of *C. ambrosioides* on Randall - Selitto assay. The results indicates that administration of different potencies (3x, 6x, 12x and 30c) of *C. ambrosioides* or alcohol at a dose of 0.5 ml/rat/day had increased the degree of threshold pressure (141.33 to 146.66 g) to mechanical induced pain on 10th day. The increase in threshold pressure was significant ($p<0.05$) in those rats treated with 6x potency. Such effect did not persist but gradually tapered off on 20th day and 30th day of experiments on further continuation of the treatment. On the other hand the quantum of threshold pressure required to elicit vocalization to applied mechanical pain was more or less same (131.33 to 133.33 g) on day 1 before administration of drug, alcohol or normal saline and 30 min after the administration of normal saline on different days of experimentation (Fig.3).



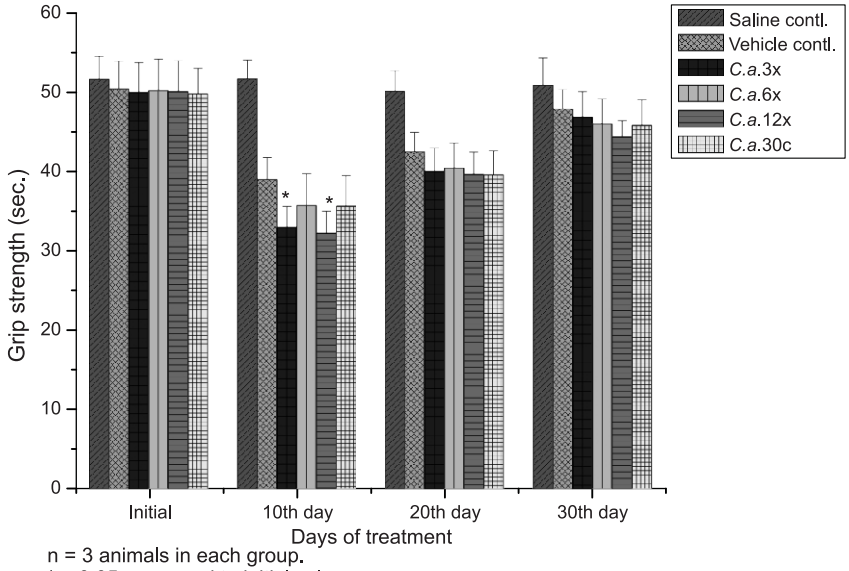

n = 3 animals in each group.
*p<0.05 compared to initial value.

Fig.3 Analgesic effect of *C.ambrosioides* (0.5ml/rat/day) on Randall - Selitto test (Mean ± S.E.M.)

## Behavioural activity

### Rota - rod test

The results of different potencies of *C. ambrosioides* on motor coordination activity of rats using grip strength test are shown in Fig.4. The results obtained from rota-rod test showed decrease (32.10 to 38.85 sec) in the grip strength of the rats when measured 30 min after the administration of the different potencies (3x, 6x, 12x and 30c) of *C. ambrosioides* or alcohol at a dose of 0.5 ml/rat/day on 10$^{th}$ day. The decrease in grip strength was significant ($p<0.05$) only with those rats treated with 3x and 12x potencies. Afterwards, there was a progressive reversal in the grip strengths of drug treated rats on further continuation of the treatment as the rats stayed for longer duration but still for less on the rota rod that was observed on day 1 (49.68 to 51.55 sec) before administration of drug when tested on 20$^{th}$ and 30$^{th}$ day of experiment (Fig.4).

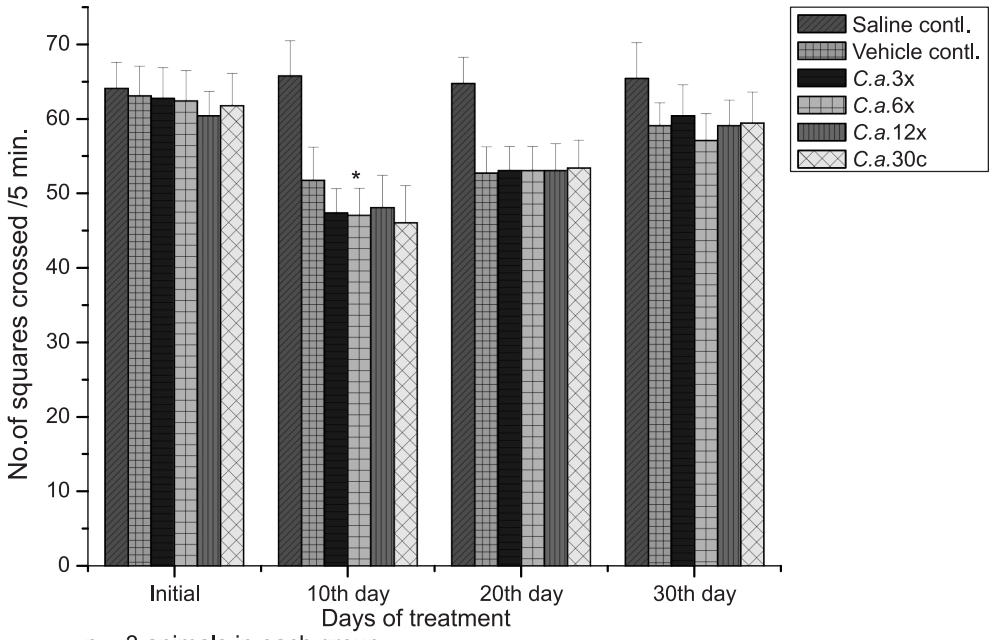



n = 3 animals in each group.
*p<0.05 compared to initial value.

Fig.4 Behavioural effect of *C.ambrosioides* (0.5ml/rat/day) on Rota - rod test (Mean ± S.E.M.)

### Open field test

The results of different potencies of *C. ambrosioides* on locomotor activity of rats by open field test are shown in Fig.5. There was a decrease in the locomotor activity of the rats (43.66 to 46.66 squares in 5 min) when measured 30 min after administration of different potencies (3x, 6x, 12x and 30c) of *C. ambrosioides* or alcohol at a dose of 0.5 ml/rat/day on 10th day of the experiment. The difference in locomotor activity was significant ($p<0.05$) with those rats treated with 3x potency when compared to initial locomotor activity taken just before administration of drug on day 1 of the study. However, such depressant effect of drug on locomotor activity slowly vanished off on continuation of drug treatment when tested subsequently on 20th and 30th day of the study. The average locomotor activity as measured in terms of crossing of the squares of a open field apparatus during 5 min of observations on day 1 before administration of drug, alcohol or normal saline and 30 min after the administration of normal saline on different days of experimentation was 'more or less' same (60.33 to 65.66 squares in 5 min) (Fig.5).




n = 3 animals in each group.
*p<0.05 compared to initial value.

**Fig.5** Behavioural effect of *C.ambrosioides* (0.5ml/rat/day) in the Open field test (Mean ± S.E.M.)

## Discussion and Conclusion

The present preliminary study was undertaken to have some assessment about the analgesic and behavioural activities of different (3x, 6x, 12x and 30c) potencies of *C. ambrosioides* in albino rats. The findings are accordingly presented.

*C. ambrosioides* in different potencies (3x, 6x, 12x and 30c) were evaluated in hot plate, ice pate and Randall - Selitto tests for analgesic activity because of the facts that these tests are very sensitive and reliable for development of analgesic drugs. The results indicated that all the four potencies (3x, 6x, 12x and 30c) of *C. ambrosioides* had increased the latency times for both thermal noxious stimulus and cold sensation and had also increased the quantum of threshold pressure to mechanical induced pain when measured on 10th day of study 30 min after the administration of the drug. On the other hand, the latency time and the quantum of threshold pressure were constant in saline treated animals. A number of flavonoids have been reported to produce analgesic activity [16]. The present result also suggests that the analgesic activity of *C. ambrosioides* could be due to the presence of flavonoids and terpenoids in the plant.

*C. ambrosioides* in different potencies (3x, 6x, 12x and 30c) were also evaluated in rota rod as well as open field tests for its behavioural activity. The present results showed that different (3x, 6x, 12x and 30c) potencies of *C. ambrosioides* had decreased the grip

strength and locomotor activity when measured on 10th day of study 30 min after the administration of the drug. Vehicle (91.5%v/v alcohol) in same dose behaved in a similar way as that of different potencies of *C. ambrosioides* for its analgesic and behavioural activities. The only different was that the said effects were comparatively less in compared to drug treated animals.

Increased in the latency time to noxious thermal stimulus and/or cold sensation and increased in the quantum of threshold pressure to mechanical induced pain and decreased locomotor activity and motor coordination by the drug is the sign of CNS depression[17,18]. Wearing off the depression on prolonged and continuous use of the drug may be either due to decreased sensitivity of the central nervous system or due to increased metabolising enzymatic activity in the liver. Alcohol used as a vehicle to prepare different potencies (3x, 6x, 12x and 30c) of the drug *C. ambrosioides* is well known to have these effects on its prolonged use.

These preliminary results are reported which suggests that the homoeopathic formulations of *C. ambrosioides* may possess CNS depressant property. However, further detailed experimental studies are warranted for arriving any meaningful conclusion so that it can have therapeutic application.

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