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RESEARCH ARTICLE

Camphora Augments Humoral Mediated Immunity and Decreases Delayed type Hypersensitivity in BALB/c Mice

Vara Prasad Saka[1], G. V. Narasimha Kumar[1*], Abanti Goswami[1], Digvijay Verma[2] and Pankaj Gupta[3]

[1]Drug Standardization Department, Dr Anjali Chatterji Regional Research Institute for Homeopathy, Under Central Council for Research in Homeopathy, Kolkata - 700035, West Bengal, India; narasimha87@ccrhindia.nic.in
[2]Drug Standardization Department, Central Council for Research in Homeopathy, New Delhi - 110058, India
[3]Drug Standardization Department, Dr D P Rastogi Central Research Institute for Homeopathy, Under Central Council for Research in Homeopathy, Noida - 201301, Uttar Pradesh, India

Abstract

Background: Immunomodulation encompasses all therapeutic interventions aimed at modifying the immune response. The immune response augments are desirable to prevent infection in immunodeficiency states and fight established diseases. In this context, the present study investigated the effect of the homeopathic medicine, *Camphora,* in 6CH, 30CH, and 200CH potencies on immunomodulation in experimental animals. The acute oral toxicity study was also carried out in 6CH, 30CH, and 200CH potencies to determine the safe dose volume for the immunomodulatory study. **Methodology:** Acute oral toxicity studies of *Camphora* in 6CH, 30CH, and 200CH potencies were carried out as per OECD guideline 423 with slight modifications in Wistar albino rats. Humoral immunity, i.e., primary and secondary humoral responses, was assessed by measuring the hemagglutination titre of sheep red blood cells. Delayed-Type Hypersensitivity (DTH) was evaluated by measuring footpad thickness in BALB/c mice. **Results:** *Camphora* in 6CH, 30CH and 200CH potencies at a dose volume of 2000 **µ**l/kg did not cause any mortality in the rats when administered as a single dose. *Camphora* in 6CH, 30CH and 200CH potencies showed augmented primary and secondary humoral responses against the SRBC antigen in BALB/c mice. However, the values were statistically non-significant except in the case of 6 CH potency ($p<0.01$), which showed statistically significant primary anti-SRBC antibodies. In the DTH assay, *Camphora* in 6CH, 30CH and 200CH potencies significantly decreased the paw volume ratio after 24 hrs of SRBC injection in the paw, thus insinuating its role in reducing cell-mediated immunity. *Camphora* in 6CH, 30CH and 200CH potencies also showed enhanced antibody titres and decreased paw volume compared to vehicle control, i.e. dispensing alcohol, suggesting that the effect was imminent because of *Camphora*. **Conclusion:** The study results indicate that *Camphora* in 6CH, 30CH, and 200 CH potencies is safe up to a dose volume of 2000 µl/kg when administered as a single dose, augments the primary and secondary humoral immunity, and decreases DTH in experimental animals. The current study's findings suggest that *Camphora* might be useful as an immunomodulator in treating immune system disorders and infectious diseases and require further investigation to investigate its mechanism of action.

Keywords: *Camphora*, Cell-mediated Immunity, Delayed Type Hypersensitivity, Homeopathic Medicine, Humoral Immunity, Immunomodulation

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1. Introduction

The quest for safer immunomodulating drugs to treat severe acute respiratory syndrome-associated coronavirus type-2 (SARS-CoV-2) infection and other infectious disorders has intensified with the rise of the COVID-19 pandemic. Immunomodulation is a notion that entails the non-specific activation of immune system components' functions and effectiveness. Non-specific activation includes instigating macrophages, granulocytes, complement, natural killer cells, and lymphocytes and synthesising various effector chemicals by activated cells. These non-specific actions protect against multiple pathogens, including bacteria, viruses, and fungi[1].

Despite the availability of a number of chemical immunomodulators on the market, which alter immune responses by stimulating, expressing, amplifying, or inhibiting any part or step of the process[2],their severe side effects necessitated the search for safer medications[3]. In recent years, there has been a surge of interest in the quest for intriguing perspective chemicals for studying immunomodulatory substances found in natural sources. Some of the therapeutic effects of plant extracts or chemicals have been attributed to their immune system effects[3].Plants have an essential role in Complementary and Alternative Medicine (CAM) because of their potential to produce secondary metabolites such as proteins, flavonoids, alkaloids, steroids, and phenolic compounds, which are used to restore health and heal a variety of ailments. Herbal medications are thought to boost the body's natural resistance to infection, and several plants have been found to have immunomodulatory properties[4].

Among CAM systems of medicine, Homeopathy is one of the widely used systems of medicine, which uses all plant parts, exudates, and processed materials as its primary sources. Homoeopathy belongs to therapeutic approaches that help the body's immune system[5,6]. Homoeopathy is well known for its ultra-diluted medications that are prepared by potentization to acquire their medicinal properties. Potentization is a process that requires serial dilutions and succussions (vigorous shaking), which the Father of Homeopathy, Samuel Hahnemann, first described[7]. In this process, 1 ml of mother tincture, or the original extract, is typically diluted with 99 ml of 90% ethyl alcohol and given ten "succussions" or "jerks" to generate the potency of 1 CH (centesimal). Similarly, 1 ml of medicine at 1 CH potency is mixed with 99 ml of 90% ethanol, and ten succussions are administered to make the potency 2CH, and the process continues. Different potencies, such as 6 CH, 30 CH, 200 CH, and beyond, are made by consecutive dilutions and succussions, as discussed above[7]. Several homeopathic medicines are in clinical use, and their efficacy in various pathological conditions has been documented by multiple preclinical[8-12] and clinical trials[13-16].

Given its clinical effectiveness in severe viral infections[17], homeopathy could be considered an adjunct to conventional care in COVID-19 patients. *Camphora* is a well-known medicine employed in the homoeopathic medicine system, made of Camphor. Camphor is a natural substance obtained by steam distillation and sublimation from the wood of *Cinnamomum camphora* L. or *Ocimum canum* Sims, containing not less than 96.0 percent of $C_{10}H_{16}O$[18,19], as shown in Figure 1. It is listed in the essential drug list and clinically used in the potentized forms of 6CH and 200CH in homeopathy[20]. According to John Henry Clarke's Materia Medica, its main therapeutic effect is to treat cold, collapse, and repercussed eruptive illnesses[21]. *Camphora* was one of the medicines used as an adjunctive treatment for patients with mild to severe symptoms of SARS-CoV-2 infection[22]. However, its exact mechanism of action in alleviating the symptoms is unknown.

Camphor is a prominent component in modern medicine for treating minor muscle aches and pains with topically applied analgesics and rubefacients. It has been claimed that camphor has been administered intramuscularly to reduce pain caused by breast engorgement. It is also a key component of liniments for alleviating bursitis and neuralgia. When swallowed, it also has irritating and carminative qualities and can be used as a mild expectorant[18,23,24].

Previous research found that the extracts and camphor oil made from *Cinnamomum Camphora* augmented pro-inflammatory cytokine production of pro-inflammatory cytokines *in vitro*[25,26]. Hypothesising that homeopathic medicine, *Camphora*, might be acting via modulating the immune system, the present study was planned to investigate the immunomodulatory effects of *Camphora*

in 6CH, 30CH, and 200CH potencies in BALB/c mice model system of humoral mediated immunity and delayed-type hypersensitivity. The oral acute toxicity study was also carried out before the immunomodulatory research to assess the effects of *Camphora* in 6CH, 30CH, and 200CH potencies in Wistar albino rats after an acute overdose to determine the safe dose volume for the immunomodulatory study.

IUPAC Name: (+) (1*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one

Figure 1. Chemical Structure of Camphor.

2. Materials and Methods

2.1 Chemicals

Camphora (6 CH, 30 CH and 200 CH) potencies and dispensing alcohol (90%) were procured from Hahnemann Publishing Co. Private Ltd. Sodium Chloride, Potassium Chloride, Sodium Phosphate Dibasic Anhydrous, Potassium Dihydrogen Orthophosphate and Sodium Hydroxide for preparing Phosphate Buffered Saline (PBS) (pH 7.2 to 7.4) and Dextrose, Sodium Citrate, Sodium Chloride and Citric Acid for preparing Alsever's Solution (pH 6.1) were procured from Sisco Research Laboratories (SRL) Pvt. Ltd. All the chemicals used in the study were of analytical grade.

2.2 Animals

Thirty healthy and nulliparous adult female Wistar albino rats of 6-8 weeks of age with 200 ± 20 gm of weight were obtained from the State Centre for Laboratory Animals, West Bengal, to carry out an acute oral toxicity study. Sixty (30 males and 30 females) BALB/c mice of 6-8 weeks of age with a weight of 20-25 g were taken from the inbred stock of in-house small animal house facility of Dr Anjali Chatterji Regional Research Institute for Homeopathy, Kolkata, for assessing the humoral and cell-mediated immunity. Animals were grouped and housed in an Individually ventilated polysulphonate caging system (M/s Citizen Industries Ltd., India) with corncob bedding (M/s Sparconn Life sciences, India) and maintained at 19 to 23 ˚C temperature, 30 to 70 % relative humidity, and 12 Hrs light and dark cycles. All the animals were fed a standard pellet diet (M/s Vishnu Traders, India) and purified RO water *ad libitum*. The IAEC approved all the protocols of Dr Anjali Chatterjee Regional Research Institute for Homoeopathy (Reg. No.: 2055/GO/RBi/S/19/CPCSEA), Kolkata (Proposal no: DACRRIH/CPCSEA/IAEC/2021/006 for acute oral toxicity study and DACRRIH/CPCSEA/IAEC/2021/005 for immunomodulatory research).

2.3 Experimental Design

2.3.1 Sample Size

The acute oral toxicity study followed OECD Guideline 423 and used 6 rats per group. Using G3 (v.3.1) software, a priori power analysis was performed to determine the sample size for the immunomodulatory activity experiment. With a power of 82 percent and an alpha error of 0.05, 10 mice per group were obtained, and the same was used in the immunomodulatory activity.

2.3.2 Blinding

Camphora 6 CH, 30 CH, 200 CH, and vehicles were partially blinded to the researchers by coding the test compounds. The treatments were decoded during the report drafting phase[27].

2.3.3 Acute Oral Toxicity Study

Acute oral toxic effects of *Camphora* in 6 CH, 30 CH, and 200 CH potencies were evaluated using OECD 423 guidelines with slight modifications. Female rats were quarantined and acclimatised for at least a week before testing the drugs. Rats were examined for health and then randomly allocated to the five groups (6 animals per group) for steps 1 and 2 evaluation. Rats were kept fasting for four hours before drug doses were administered, with access to water *ad libitum*. Group I served as the normal control and received distilled water; group III received 90% dispensing alcohol, and groups V, IV, and II received *Camphora* in 6 CH, 30 CH, and 200 CH potencies, respectively, at a dose of 2000 µl/kg body weight. The experimental procedure is depicted in Figure 2. The following observations were made during the period of study:

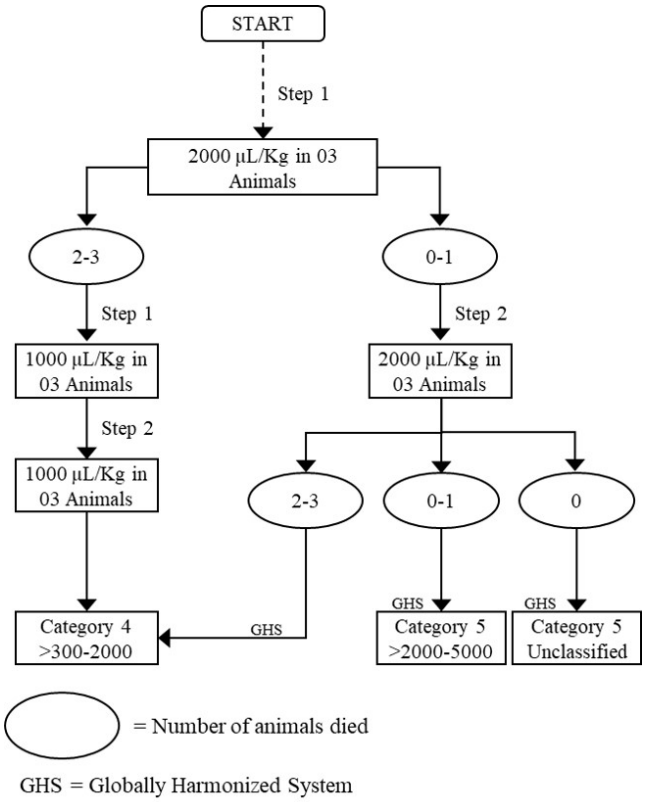


Figure 2. Steps involved in the oral acute toxicity study.

2.3.3.1 Clinical Signs

All the animals were examined for clear clinical signs at the 0th minute (before dosing), 30 minutes, 1hr, 2hr, 3hr, 4hr, and 24hr, with a difference of ± 5 to 10 minutes after the dose administration. Changes in physical observations, behavioural observations, posture, appendages, sensory responses, reflexes, autonomic effects, and respiratory effects were monitored and recorded in their appearance and disappearance. Mortality and moribundity were also observed for 14 days.

2.3.3.2 Body Weight

The animals were weighed individually on day 1 (before dosing), day 7 and day 14 before the necropsy. The results were expressed as mean body weight (g) and percentage (%) change in body weight compared to Day 1[28].

$$\%\ Body\ Weight\ Change = \frac{Day\ 1 - Day\ 14}{Day\ 1} \times 100$$

2.3.3.3 Relative Organ Weight and Gross Necropsy

Necropsy was done in acute oral toxicity test groups of animals on day 15 after sacrificing the rats by CO_2 euthanasia. The external surface of the body, orifices (anal, urethral, vaginal, and nasal), and cavities (thoracic and abdominal cavities) were observed. The vital organs (brain, lungs with trachea, heart, thymus, liver, gastrointestinal tract, spleen, adrenals, kidneys, ovaries, and uterus with cervix) were removed through a midline incision in the rat's abdomen. The organs were cleaned of fat, blotted with clean tissue paper, and then weighed on a balance. The relative organ weight was calculated and recorded in proportion to the body weight using the following equation[28].

$$\%\ Relative\ Organ\ Weight = \frac{Absolute\ Organ\ weight}{Body\ weight\ at\ sacrifice} \times 100$$

2.3.4 Immunomodulatory Activity

2.3.4.1 Preparation of Antigen

Fresh sheep blood was collected from the local slaughterhouse for Alsever's solution. The sheep red blood cells (SRBC) were washed three times with phosphate-buffered saline (PBS) and adjusted to a concentration of 1 × 10^8 cells/ml for further studies[29].

2.3.4.2 Immunisation and Treatment

10 (05 males and 05 females) BALB/c mice were randomly assigned to each group and equally distributed into six groups. Groups I and II served as normal control and SRBC control, respectively, and received distilled water from day 1. Group V served as vehicle control and received dispensing alcohol (90% alcohol), while groups III, VI, and IV received *Camphora* potencies 6 CH, 30 CH, and 200 CH, respectively. All the interventions were given at a dose of 400 µL/Kg body weight diluted with distilled water in a 1:9 ratio[30,31] orally daily for 27 days. On days 14 and 21, all groups except group I received 0.5 ml of 5 × 10^8 SRBCs into the intraperitoneal cavity of each animal. On days 21 and 27, blood was withdrawn from the mice through the retro-orbital plexus, and serum was isolated to assess primary and secondary humoral antibody responses to SRBC[29,32].

2.3.4.3 Hemagglutinating Antibody (HA) Titre: Primary HA Titre and Secondary HA Titre

The method used was similar to that described previously by Puri et al. (1993). On the 21st and 27th days of the treatment, primary and secondary antibody titres were determined by titrating serum dilutions with a 25µl quantity of a new 1% SRBC suspension in PBS. The microtiter plates were incubated at 37°C for 24 h and examined visually for agglutination. The reciprocal of the highest number dilution of serum showing Haemagglutination is expressed as HA titre[29].

2.3.4.4 Delayed Type Hypersensitivity (DTH) Assay

After the blood-serum collection from animals on the 27th day of treatment, 25 µL of SRBC suspension in sterile PBS, containing 1×10^8 SRBCs, was injected into the right hind footpad. The left hind footpad was injected with sterile PBS. The footpad volume was measured with a digital Plethysmometer (Orchid Scientific and Innovative India Pvt. Ltd.) before and after 24 hours of injecting SRBC[29,32,33].

2.4 Statistical Analysis

All data were expressed as the means ± SEM. One-way ANOVA and Tukey's multiple comparison test were performed for comparisons between various groups. SPSS software (version 26) was used for all statistical analyses. Values of $p<0.05$ were considered the threshold for statistical significance.

2.5 Reporting

The study protocol and report were prepared following PREPARE and ARRIVE guidelines, respectively[34,35].

3. Results

Camphora in 6 CH, 30 CH, and 200 CH potencies were tested for acute oral toxicity, humoral toxicity, and delayed-type hypersensitivity in experimental animals.

3.1 Acute Oral Toxicity Study of *Camphora* 6 CH, 30 CH, and 200 CH Potencies

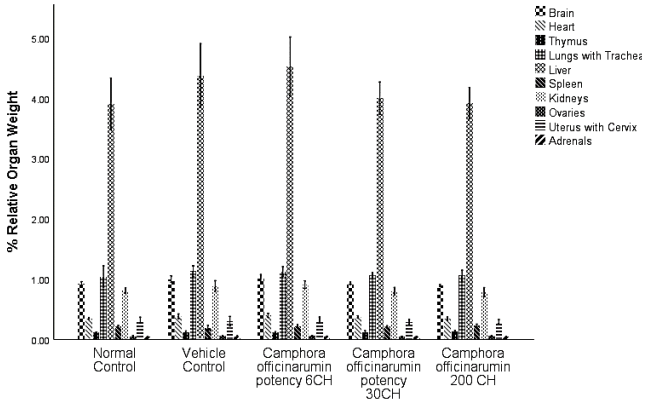
In the oral acute toxicity study, the following observations were made to assess the safety of *Camphora*:

3.1.1 Clinical Signs

6 CH and 30 CH Potencies and dispensing alcohol-treated animals showed non-persistent ataxia and mild changes in the limb position, which recovered within 3 hours of the administration of treatments. There were no further anomalies in the clinical symptoms of any of the potencies. All the animals appeared to be normal. The observed data are presented in Table 1.

3.1.2 Bodyweight, Mortality, Moribundity, and Gross Necropsy

The results indicate no significant changes in the body weight of rats across all groups. No signs of mortality and moribundity were observed in any treated animals. Compared to the normal group, no significant change was observed in relative organ weights (Figure 3). Gross necropsy revealed that the treated animals' outer surface, cavities, orifices, and organs were normal when compared to the normal control group. The observed data and photographs of organs during gross necropsy are presented in Tables 1 and 2, respectively.



Data are shown as mean + SEM, n = 06. Statistical analysis was carried out employing the ANOVA followed by the Tukey test.

Figure 3. Effect of *Camphora* on relative organ weights of female albino Wistar rats.

Table 1. Clinical signs exhibited by *Camphora* in experimental animals during acute oral toxicity study

S. No	Group	Intervention	% change in body weight	Mortality (n/N)	First appearance of the clinical sign among animals of the group (n/N)	The disappearance of the clinical sign from all the animals in the group
1.	I	Normal Control	4.80 ± 0.66	0/6	No clinical sign was observed (0/6)	No clinical sign was observed (0/6)
2.	II	*Camphora* potency-200CH	5.11 ± 0.63	0/6	No clinical sign was observed (0/6)	No clinical sign was observed (0/6)
3.	III	*Camphora* potency-30CH	4.83 ± 0.66	0/6	Ataxia at 30 (±5) minutes observation (2/6)	Ataxia at 2hr (±10) minutes observation
4.	IV	Vehicle control alcohol	4.66 ± 0.49	0/6	Ataxia at 30 (±5) minutes observation	Ataxia at 2hr (±10) minutes observation
5.	V	*Camphora* potency-6CH	5.16 ± 0.46	0/6	Ataxia at 30 (±5) minutes observation (2/6)	Ataxia at 2hr (±10) minutes observation

(n/N) = no of animals affected / total no of animals per group; Data presented in mean ± SEM; n = 6

3.2 *Camphora* Increases Humoral Immune Response to SRBC in BALB/c Mice

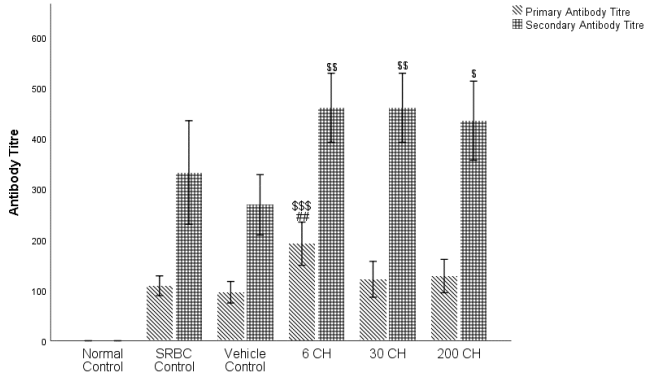
3.2.1 Primary HA Titre

The 21-day HA titre/primary antibody titre analysis revealed that the *Camphora* in 6 CH ($p<0.01$) had a statistically significant impact on elicitation of the humoral immune response following immunisation with sheep red blood cells (SRBCs) in BALB/c mice compared to SRBC control group. While 30 CH and 200 CH potencies also improved the primary antibody titre, the values were statistically nonsignificant.

3.2.2 Secondary HA Titre

Camphora in 6 CH, 30 CH, and 200 CH potencies had shown augmentation of the humoral immune response following a booster dose with SRBCs in BALB/c mice on the 27th day. However, responses were statistically non-significant compared to the SRBC control group.

As evident from Figure 4, primary and secondary antibody titres were significantly higher in *Camphora* at 6 CH, 30 CH and 200 CH potencies than in the vehicle control group, i.e., when dispensing alcohol at -90%. Thus, the effect can be attributed to *Camphora*.



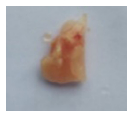
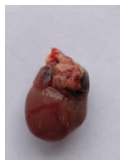
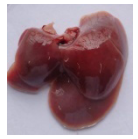
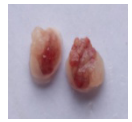
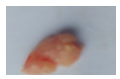
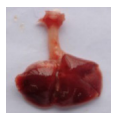
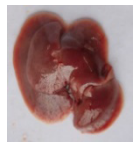
Data are shown as mean ± SEM, n=10. Statistical analysis was carried out employing the ANOVA followed by the Tukey test. ##: $p<0.01$ compared with SRBC Control; $$: $p<0.01$, $$$: $p<0.001$ compared with Vehicle Control.

Figure 4. Effect of *Camphora* on primary and secondary humoral immune response in BALB/c mice.

3.2.3 Difference between HA-Titre on Days 21 and 27

Camphora 6 CH, 30 CH and 200 CH potencies had shown an increase in HA-titre between the 21st and 27th days of the treatment, indicating an augmented secondary humoral immune response shown in Figure 5.

Table 2. Gross necropsy pictures of different organs of experimental animals in the acute oral toxicity study

Group	Interventions	Brain	Lungs With trachea	Thymus	Heart	Liver	Spleen	Adrenals	Kidneys	Ovaries	Uterus With cervix
I	Normal control distilled water										
II	*Camphora* in potency-200 CH										
III	*Camphora* in potency-30 CH										
IV	Vehicle control alcohol										
V	*Camphora* in potency-6 CH										

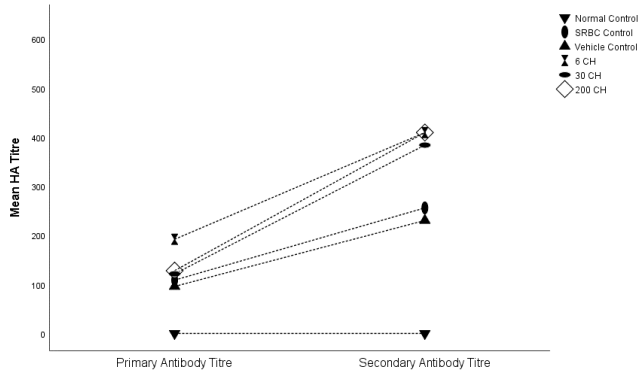
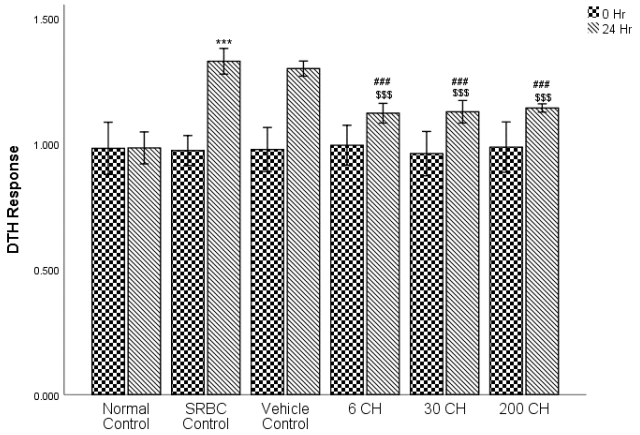


Figure 5. Difference between primary and secondary HA-Titre in BALB/c mice at days 21 and 27 treated with *Camphora* 6CH, 30CH and 200 CH potencies.

3.3 *Camphora* Decreases Delayed-type Hypersensitivity (DTH) Response in BALB/c Mice

In this study, we found an increase in paw volume (the right/left ratio was taken for comparison between groups) in all the groups compared to the normal control group after 24 hours of injection, indicating that SRBC had caused local inflammation. After 24 hrs, the animals treated with 6 CH ($p<0.001$), 30 CH ($p<0.001$), and 200 CH ($p<0.001$) potencies significantly decreased their paw volume ratio when compared to the SRBC control, suggesting their role in reducing DTH response. The effect of *camphor* in 6 CH, 30 CH, and 200 CH potencies was also statistically significant compared to vehicle control after 24 hrs. Figure 6 represents the paw volume measurements in the study.



Data are shown as Mean ± SEM, n=10. Statistical analysis was carried out employing the ANOVA followed by the Tukey test. ***: $p<0.001$ compared with Normal Control; ###: $p<0.001$ compared with SRBC Control; $$$: $p<0.001$ compared with Vehicle Control.

Figure 6. Effect of *Camphora* on cell-mediated immune response/DTH test in BALB/c mice.

4. Discussion

Camphora is a widely used homeopathic medicine to treat cold, congestion, and the initial stages of respiratory infections. *Camphora* was also one of the medications used as an adjunctive treatment for patients with mild to severe SARS-CoV-2 symptoms[21,22]. Safety is a significant concern in the present scenario, considering the massive demand for Homeopathic medicines[36]. Moreover, preclinical reports on the safety evaluation of *Camphora* in 6 CH, 30 CH, and 200 CH are unavailable. Hence, in the present investigation, *Camphora* was evaluated for its safety in its potencies 6 CH, 30 CH, and 200 CH as per OECD guidelines 423[37] with minor modifications. This test was conducted on female albino Wistar rats at a maximum dose of 2000 µL/Kg body weight. The tested dose level was safe since mortality or moribundity was not observed in any of the treated animals during the 14-day observation period. Though there was ataxia in drug- and vehicle-treated animals, it disappeared over time, as indicated in Table 1. The non-persistent ataxia was also seen in the vehicle control group; hence, the effects can be attributed to dispensing alcohol. The results indicate that the median lethal dose (LD_{50}) of *Camphora*, in 6CH, 30CH, and 200CH potencies, upon a single oral administration to female Wistar rats, was greater than 2000 µl/kg bodyweight. Further, for evaluating the effect of *Camphora* on humoral-mediated immunity and delayed-type hypersensitivity in BALB/c mice, 1/10th of LD_{50}, i.e., 400 µl/kg body weight, was selected after converting the dose from rats to mice using virtual web-based software developed by Janhavi *et al.*[38] The 400 µl/kg dose of *Camphora* was diluted with distilled water in a 1:9 ratio to mitigate the effects of the alcohol used as a vehicle in *Camphora*[30,31].

The immune system is one of the defence mechanisms of the human body. Upon activation, it effectively protects from the invasion of foreign organisms and is thus essential to maintaining the body's homeostasis. Among the immune responses, the adaptive immune response indicates the diversity, specificity, memory, and self-recognition of antigen-specific lymphocytes among the leukocytes. Immunomodulators are substances that either stimulate or suppress the immune system to control the symptoms of an infectious disease. Therefore,

immunomodulators have gained global importance in managing disease conditions[39,40]. Hence, the present study was planned to investigate the immunomodulatory effect of homeopathic medicine *Camphora* in 6 CH, 30 CH, and 200 CH potencies in BALB/c mice model system of humoral mediated immunity and DTH response.

Humoral immunity involves the interaction of B cells with the antigen and their subsequent proliferation and differentiation into plasma cells that secrete antibodies. Antibodies thus function as the effectors of the humoral response by binding to the antigens and neutralising them or facilitating their elimination by cross-linking to form clusters that are then ingested by phagocytic cells[39]. Both endogenous variables and cellular interactions influence the magnitude of the humoral response. During the response, macrophage recruitment is required for antigen processing and presentation. In contrast, T-lymphocytes are required for B-lymphocyte transformation into antibody-secreting plasma cells during T-dependent antigen processing. T-cell-dependent antigens, such as Sheep Red Blood Cells (SRBC), were used as an antigenic agent in this study41. The HA titer was determined to evaluate the effect of *Camphora* in its potencies of 6 CH, 30 CH, and 200 CH on the humoral immune response in BALB/c mice. In the case of the primary humoral immune response, 6 CH potency ($p<0.01$) showed significant primary anti-SRBC antibody titre compared to SRBC control. *Camphora* had also increased anti-SRBC antibody titre values in the case of secondary humoral immune response compared to the SRBC control, but the values were statistically non-significant. The anti-SRBC antibody titre values in the secondary humoral immune response were high compared to values in the primary humoral immune response, suggesting an immunostimulant effect of *Camphora*. The augmentation of humoral immune response to SRBC by *Camphora* may indicate the enhanced responsiveness of macrophages and both T- and B-lymphocyte subsets involved in antibody synthesis by acting as an immunostimulant.

The DTH reaction is characterised by an immuno-inflammatory response, with macrophages and Th1 cells prominent. DTH is involved in graft rejection, tumour immunity, and immunity to various intracellular pathogenic bacteria, including those that cause chronic illnesses like tuberculosis. It requires activated T cells to recognise a specific antigen and then proliferate and produce cytokines in response[42,43]. The DTH assay represents an exciting tool to monitor the *in vivo* disposition of T cells from a given individual toward specific antigens. It is valuable for two reasons, i.e., it not only monitors recalled T cell responses but can detect both pro-inflammatory and anti-inflammatory T cell responses. This allows it to determine whether and how a given individual has recognised and responded to specific antigens[44]. The present study results indicate that *Camphora* in 6 CH, 30 CH and 200 CH potencies significantly decreased the paw volume ratio after 24 hrs of SRBC injection. A decrease in the paw volume suggests that *Camphora* might act as an anti-inflammatory agent, probably by inhibiting cell recruitment at the site of SRBC injection, thus insinuating its role in decreasing cell-mediated immunity[41].

Further details could be unveiled by examining the immune cell recruitment at the site of injection of SRBC. In addition, *Camphora* in 6 CH, 30 CH and 200 CH potencies significantly enhanced antibody titres and decreased paw volume compared to vehicle control, i.e., dispensing alcohol, suggesting that the effect was imminent because of *Camphora*. The results of the current study warrant further investigation of the in-depth molecular pathways by which *Camphora* modulates the immune system *in vivo,* which are under investigation.

5. Conclusion

In conclusion, the current investigation of homeopathic medicine, *Camphora* in 6 CH, 30 CH, and 200 CH potencies in BALB/c mice demonstrates that the treatment enhances primary and secondary humoral immune responses while decreasing delayed-type hypersensitivity response. *Camphora* in 6 CH, 30 CH, and 200 CH potencies was also found to be safe up to a dose of 2000 µL/kg body weight of rats upon single oral administration. The current study's findings suggest that *Camphora* may be effective in treating immune system disorders and infectious diseases as an immunomodulator. Further investigation of the in-depth molecular pathways by which *Camphora* modulates the immune system *in vivo* in viral infections, autoimmune disorders, inflammatory conditions, and allergies is warranted, and is under investigation.

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7. Ethical Statement

IAEC approved all the experiments conducted on animals of Dr Anjali Chatterjee Regional Research Institute for Homoeopathy (Reg. No.: 2055/GO/RBi/S/19/CPCSEA), Kolkata (Proposal no: DACRRIH/CPCSEA/IAEC/2021/006 for acute oral toxicity study and DACRRIH/CPCSEA/IAEC/2021/005 for immunomodulatory research).

8. References

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