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EXTRA MURAL RESEARCH

Search for Potential Anticancer Agents:

Evaluation of Anticancer Activity of Carcinosin, Apis and Thuja

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Abstract

As a part of our composite programme of rational drug discovery, homeopathic medicines *Carcinosin, Apis and Thuja* with different strengths were taken into consideration for anticancer activity to find out the effectiveness of these drugs in animal models. Here, we have reported the anticancer activity of Carcinosin of some strengths from a manufacturer where tumor cell count as well as tumor weight inhibition were considered as the biological activity parameters. Anticancer activity of all collected medicines of Carcinosin, Apis and Thuja were evaluated against another animal model considering the survival time as the biological activity parameter. Result showed that the survival time of Carcinosin is proportionally increased with potency and the observed result also satisfies the validity of the animal model for anticancer evaluation as well as supports our earlier observations. Apies and Thuja did not show promising result to increase the survival time.

Keywords : apis, carcinosin, thuja, ehrlich ascites carcinoma, survival time

Introduction

As a part of our composite programme of rational drug discovery[1-21], homoeopathic medicines were taken into consideration as complementary medicines. Anticancer evaluation of different strength of Carcinosin, Apis mel. and Thuja occ. were reported previously[4,11,21]. It was found that the anticancer activity of Carcinosin increased with dilution, i.e., potency of Carcinosin increases anticancer activity[11]. Carcinosin 200 and Carcinosin 1M showed good anticancer activity in the animal model[6] but Apis mel. and Thuja occ did not show any significant anticancer activity in that animal model[4]. There was no similarity between the anticancer activity and the potency of samples of Apis mel. and Thuja occ[6]. While working with Carcinosin, to quantify its potential as an anticancer agent in Ehrlich Ascites Carcinoma (EAC) cells, we observed certain discrepancies in the actual content of active constituents in the preparations available in the market and we reported those findings[12,21]. The incomplete specifications in the Homeopathic Pharmacopoeia[22] in this area encouraged us to characterize these homeopathic medicines before going for their further evaluation of biological activity. Mother tinctures of *Thuja occidentalis*, *Apis mellifica*, *Lycopodium clavatum* and *Hydratis canadensis* were chosen for that study. It was found that because of the discrepancy in the drug contents of various marketed mother tincture preparations, it was wise to make dilutions from their accurately measured drug contents and study their anticancer potential against EAC cells and these part of the work was also reported[12].

After characterization of the mother tincture of Thuja, Apis, Hydratis and Lycopodium, specific quantity of residues of these after evaporation of solvents were evaluated for their anticancer activity against EAC cells in Swiss Albino mice[4]. Anticancer activities of orally administered *Thuja occidentalis*, *Apis mellifica*, *Lycopodium clavatum* and *Hydratis canadensis* obtained from different manufacturers like Hahnemann Publishing Co. Pvt. Ltd. (India), C Ringer & Co. (India), Dr. Willmar Schwable India Pvt. Ltd. (India), Dr. Willmar Schwable Karlsruhe (Germany) and SBL Pvt. Ltd. (India) were also reported[4]. Anticancer activities of the same administered intraperitoneally were also reported[4]. It was found that, though, the anticancer activity of *Thuja occidentalis*, *Apis mellifica*, *Lycopodium clavatum* and *Hydratis Canadensis* were very less but the oral route was more effective than the intraperitoneal one. Hence, supported the route of administration of Homoeopathic drugs in complementary medicines[4].

In traditional medicines, Carcinosin is a very common drug used for the treatment of cancer. Alhough the

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anticancer activity of Carcinosin was evaluated initially[4,6, 11, 21] but to study effects of the product (Carcinosin) of different strengths from a different manufacturer (Dr. Reckeweg & Co., Germany) were taken for the test in this part of the work based on those earlier findings to get a clear broad picture of this kind of homeopathic anticancer drug. Our aim was to search for the potential anticancer agents and here anticancer activity of potentised and dynamised Carcinosin, Thuja and Apies were evaluated against Ehrlich Ascites Carcinoma (EAC) cells in Swiss Albino Mice and were reported as a continuation of our earlier reported work[4, 6, 11-12, 21]. In comparison to our previous study[6] on anticancer activity of Carcinosin from a different manufacturer (Hahnemann Publishing Co. Pvt. Ltd., India), it was found that there was a similarity in anticancer activity of the same potencies of Carcinosin and biological activity increases with the potency. Result also satisfied the validity of this animal model for anticancer evaluation of homeopathic drugs.

Here, we have reported the anticancer activity of Carcinosin of different strengths from a different manufacturer than the earlier reported ones where tumor cell count as well as tumor weight inhibition were considered as the anticancer activity parameters. Attempt was also made to find out effects of homeopathic medicines on cancer in respect to the survival time of animals to validate our earlier work of homeopathic medicines on animal experimentation.

Materials and Methods

Carcinosin was obtained from Dr. Reckeweg & Co. (Germany). Apis mel and Thuja were manufactured by Hahnemann Publishing Co. Pvt. Ltd. (India).

Evaluation of anticancer agent

Carcinocin was evaluated for anticancer activity *in vivo* against Ehrlich Ascites Carcinoma (EAC) cells in Swiss Albino mice using EAC fluid (tumor) weight and EAC cell count as the biological activity parameters. Amongst various *in vivo* evaluation systems in practice, this method has been standardized and reported from our laboratory earlier [4, 6, 9-11, 13, 16-17, 19-21].

Survival time determination

Two groups of Swiss Albino mice, each containing 8 healthy mice of the same sex, approximately of the same age and body weight (18-20g) were selected at random and kept in two different cages under identical conditions. One of these two groups served as the control while the other as the test. EAC cells were collected from the donor mouse and were resuspended in sterile isotonic solution. The numbers of tumor cells per ml of this suspension were counted under a microscope with the help of a haemocytometer. A definite number (about 2 X 10^6 cells/0.2 ml) of these living viable cells was injected or implanted into the peritoneal cavity of each mouse. In this instance, the tumor cells multiplied relatively freely within the peritoneal cavity and ascites developed. A day of incubation was allowed to establish the disease in the body before starting the drug administration. From the 2nd day of transplantation up to the 8th day, different strengths of drugs were given from a solution of two drops in 10 ml distilled water to each mouse orally (buccal cavity was soaked properly) in the test group at 24 hr interval. Thus, seven consecutive dosage of the drug were administered to each mouse in the test group. Mice were inspected daily for survival time for the period of 30 days. Animals were also inspected for the change in their body weight every alternate day over this period.

Results and Discussion

Carcinosin 6, Carcinosin 30, Carcinosin 200 and Carcinosin 1000 were evaluated for their anticancer activity in vivo against Ehrlich Ascites Carcinoma (EAC) cells in Swiss Albino mice using EAC fluid (tumor) weight and EAC cell count as activity parameters. In comparison between % inhibition of ascitic cells and % inhibition of ascitic fluid it was found that there was no similarity. Even in case of inhibition of ascites fluid, it was found that with the increase in the dilution, anticancer activity increased accordingly for Carcinosin 6, 30 and 200 but Carcinosin 1M does not showed the expected result which is shown in Table 1. It may be due to the nonuniformity of the actual content of active constituents in the preparation available in the market as reported by our earlier work[11].

We have also check effects of the homeopathic medicines Carcinocin, Apis and Thuja on cancer in respect to survival time in cancer. The results are shown in Table 2, Table 3 and Table 4 respectively.

Conclusion

In search of potential anticancer agent, anticancer evaluation was performed on different marketed homeopathic medicines. Different homeopathic medicines were collected from different companies like a homeopathic manufacturing company from West Bengal, i.e., Hahnemann Publishing Co.Pvt.Ltd., an Indian manufacturing company, i.e., C. Ringer and Co. (India), a German manufacturing company, i.e., Dr. Willmar Schwable Karlsruhe (Germany) and a US based company, i.e., BT, USA. In the anticancer evaluation of different strengths of Carcinosin of various manufacturers, it was found in our previous report[11] that products from some companies showed good anticancer

Table 1: Anticancer activity of Carcinosin 6, Carcinosin 30, Carcinosin 200 and Carcinosin 1M against EAC cell line in Swiss Albino mice administered orally

Samples	No. of animals in each group	No. of EAC cells innoculated	Avg. no. of ascitic cells/ml in Control (C)	Avg. no. of ascitic cells/ml in Test (T)	% Inhibition of ascitic cells (1-T/C) x100	Avg. wt of ascitic fluid in Control (C)	Avg. wt of ascitic fluid in Test (T)	% Inhibition of ascitic fluid (1-T′/C′) x100
Carcinosin 6	8	$2.04x\ 10^6$	$50.98x10^6$	$27.15\ x\ 10^6$	46.74	0.82	0.72	11.87
Carcinosin 30	8	$2.04x10^6$	$50.98x10^6$	$33.59\ x\ 10^6$	34.11	0.82	0.67	17.99
Carcinosin 200	8	$2.10x10^6$	$41.60x10^6$	$39.39\ x\ 10^6$	5.31	1.85	1.19	35.68
Carcinosin 1000	8	$2.10x10^6$	$41.60x10^6$	$29.19\ x\ 10^6$	29.83	1.85	1.55	16.22

Table 2: Observation of survival time against Ehrlich Ascites Carcinoma cells of Carcinosin

Group	No. of animals in each group	No. of EAC cells inoculated	After 15 days No. of live animal	After 30 days No. of live animal
Control	8	$2.03\ x\ 10^6$	1	0
Carcinosin 6	8	$2.03\ x\ 10^6$	4	0
Carcinosin 200	8	$2.03\ x\ 10^6$	2	0
Control	8	$2.02\ x\ 10^6$	2	0
Carcinosin 30	8	$2.02\ x\ 10^6$	2	0
Carcinosin 1M	8	$2.02\ x\ 10^6$	6	3

Table 3: Observation of survival time against Ehrlich Ascites Carcinoma cells of Thuja

Group	No. of animals in each group	No. of EAC cells inoculated	After 15 days No. of live animal	After 30 days No. of live animal
Control	8	$2.12\ x\ 10^6$	2	0
Thuja 30	8	$2.12\ x\ 10^6$	1	0
Thuja 200	8	$2.12\ x\ 10^6$	2	0

Table 4: Observation of survival time against Ehrlich Ascites Carcinoma cells of Apis

Group	No. of animals in each group	No. of EAC cells inoculated	After 15 days No. of live animal	After 30 days No. of live animal
Control	8	$2.05\ x\ 10^6$	2	0
Apis 30	8	$2.05\ x\ 10^6$	3	0
Apis 200	8	$2.05\ x\ 10^6$	2	0

activity in the animal model where cell count and tumor weight were considered as the biological activity parameters. Anticancer activity of Carcinosin increased with dilutions, i.e., potency of Carcinosin increased anticancer activity but other products from some other companies did not showed the expected activity with dilutions, even there were discrepancies in results between these two parameters, i.e., % inhibition of cell count and the % inhibition of tumor weight of the ascitic fluid. It may be due to the nonuniformity of the actual content of active constituents in the preparations available in the market. There is incomplete specification in Homeopathic Pharmacopoea[22] which encourages us to determine the % content of active constituents and we have reported previously[12] the discrepancy in the drug contents of various marketed mother tinctures of Homeopathic preparations. Thus, it is better to make dilutions from their accurately measured drug contents and evaluation of anticancer activity of these ones. Due to non-availability of the mother tincture of Carcinosin, we evaluated anticancer activity of various dilution of Carcinosin available from different manufacturers. We had also checked the effect of the homeopathic medicines Carcinosin, Apis and Thuja on cancer in respect to survival time in another animal model. It is also observed that for Carcinosin 1M, survival time was the highest. Other anticancer homoeopathic medicines like Apis mel. and Thuja occ did not show any significant anticancer activity in the animal model so far survival time was considered as the biological activity parameter. There was no similarity between the anticancer activity and potency of samples of these Apis mel. and Thuja occ. This preliminary study showed the importance of animal experiments for homeopathic medicines and demands detail study in future.

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