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Potential Antitumour Activity of *Gmelina asiatica* Aerial Parts Against Dalton Ascites Lymphoma in Mice

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The aim of the present study is to evaluate the effect of chloroform extract of aerial parts of *Gmelina asiatica* (CGA) against Dalton's Ascitic Lymphoma (DAL) in Swiss Albino mice. Dalton's Ascitic Lymphoma cells were injected intraperitoneally (10^6 cells) to the mice. Two days after cells injection the animals were treated with 200 and 400 mg/kg of CGA for 14 days. Five-fluorouracil (20 mg/kg) was used as reference drug. On day 15, cancer cell number, packed cell volume, decrease in tumour weight of the mice, increase in life span and hematological parameters were evaluated and compared with the same parameters in control. A significant increase in the life span and a decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with chloroform extract of *Gmelina asiatica*. The hematological parameters were also normalized by chloroform extract of *Gmelina asiatica* in tumour-induced mice. These observations are suggestive of the protective effect of chloroform extract of *Gmelina asiatica* against Dalton's Ascitic Lymphoma.

Key Words: *Gmelina asiatica* L, Dalton's Ascitic Lymphoma, Anticancer agents.

INTRODUCTION

Cancer is a worldwide problem and is emerging as a major killer of modern era[1]. It is important to emphasise that carcinogenesis is a multi-stage process including induction, development and propagation[2,3]. The chemotherapeutic agents though effective against various types of tumour are not totally free from side effects[4]. This fostered our attempts to evaluate some plant products against cancer, as they are less likely to cause serious side effects.

Gmelina asiatica L (Verbenaceae) popularly known as Nilakkumil in Tamil and Gopabhandra in Sanskrit is a large straggling shrub found in South India. The roots are used against gonorrhea, catarrh of the bladder, rheumatism and as a blood purifier[5]. *Gmelina asiatica* is claimed to be useful in rheumatism, since it possess antiinflammatory nature[6]. The root of the plant has potent hypoglycemic activity also[7]. This study was planned to evaluate the effect of chloroform extract of aerial parts of this plant against Dalton's Ascitic Lymphoma (DAL).

EXPERIMENTAL

Aerial parts of *Gmelina asiatica*, were collected during February 2008 from Therkkumalai Estate, Courtallam Hills, Western Ghats of Tamil Nadu and the identification and authentication of the plant were carried out by Dr. V. Chelladurai, Research Officer and Central Council for Research in Ayurveda and Siddha, Palayamkottai. Voucher specimen was prepared and preserved in the herbarium of K.M. College of Pharmacy, Madurai for future reference (Voucher specimen No. KMCP/GA/23). The dried powdered aerial parts of the plant (500 g) were defatted using petroleum ether and subjected to subsequent extraction in a Soxhlet apparatus using chloroform. The solvents were recovered from the respective extracts under reduced pressure to obtain a semisolid mass and vacuum dried to yield solid residues. The chloroform extract of *Gmelina asiatica* was named as CGA and it was chosen for further study.

Test animals: Male Swiss albino mice (10 weeks-old, 20-25 g) were used for testing the anticancer activity. Institutional Animal Ethics Committee has approved the project (160/1999/CPCSEA). The animals were housed at central animal house (Raja Muthiah Medical College and Hospital, Annamalai University, Tamilnadu, India) under standard conditions of temperature (27 ± 2 ºC), relative humidity (44-56 %) and light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with standard diet (Lipton, India) and water *ad libitum*. All the experiments were performed in the morning according to the ethical guidelines for the care of the laboratory animals.

Induction of cancer using DLA cells: Dalton's Lymphoma Ascites (DLA) cells were supplied by Amala cancer research centre, Trissur, Kerala, India. The cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation. While transforming the tumour cells to the grouped animal the DLA cells were aspirated from peritoneal cavity of the mice using saline. The cell count was done and further dilutions were made, so that total cells should be 1×10^6 cells/mL/mouse. This volume was given intraperitoneally and the tumour was allowed to grow in the mice for minimum of 7 days before starting the study.

Acute toxicity studies with *Gmelina asiatica*: Acute oral toxicity of chloroform extracts of *G. asiatica* was carried out as per OECD-423 guidelines. The albino mice was fasted over night provided only water, after which the chloroform extract of *Gmelina asiatica* (GA) was administered by gastric intubations to the relevant animals orally at the dose of 5 mg kg^{-1} body weight. The animals were then observed for 14 days and maintained with normal food. A mortality rate of 2 or 3 at 14 days was recorded as a toxic dose. But when mortality was observed in one animal, then the same dose was repeated again for confirmation. However, if mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2,000 mg kg^{-1} body weight. Toxic symptoms for which the animals were observed for 72 h including behavioural changes, locomotion, convulsions and mortality. The extract was found to be safe up to 2000 mg/kg of body weight.

***In vivo* antitumor activity model using DLA cell lines:** The animals will be acclimatized to the laboratory conditions and divided into five groups *viz.* G1, G2, G3, G4 and G5 of 6 animals each and used for the study. The DLA cells injected intraperitonially (10^6 cells/mouse) to all the mice of the four groups. G1 serves as normal control, G2 serves as cancer control and was not allocated any treatment after inoculation with DLA cells. From the second day onwards the animals of G3 will be treated with 5-flourouracil (20mg/kg) while the mice of G4 and G5 will be treated with the plant extract of *Gmelina asiatica* (200 and 400 mg kg^{-1}) and the treatment continued for the next 14 days.

After the last dose, all mice from all the groups were sacrificed. The blood was withdrawn from each mouse by retro orbital plexus method and the following parameters were analyzed.

Hematological parameters: (a) WBC count, (b) RBC count, (c) Hb content, (d) Platelet count, (e) Packed cell volume.

Serum enzyme and lipid profile: (a) Cholesterol, (b) Triglycerides, (c) AST, ALP, ALT.

Derived parameters: (a) Body weight, (b) life span, (c) cancer cell count.

All biochemical investigations were done by using COBAS MIRA PLUS-S autoanalyser from Roche Switzerland.

Hematological tests were carried out in COBAS MICROS OT 18 from Roche. Newly added Hi-tech instruments MAX MAT was used for an auto analyzer for all biochemistry investigation in blood sample.

Effect of CGA on solid tumour: A separate population consisting of 18 mice was used for this study. The animals were divided into 3 groups of 6 each. Tumour cells (DLA 1×10^6 cells/mL/mouse) were injected into the right hind limb (thigh) of all the animals intramuscularly. The mice of Group-1 served as tumor control. Group-2 received GA 200 mg kg^{-1} and Group-3 received GA 400 mg kg^{-1} GA for 5 alternative days. The tumour mass was measured from the 11th day of tumour induction; diameter of tumor mass was measured on every 5th day for a period of 30 days using vernier caliper and the volume of tumor mass was calculated using the formula $r = 4/3 \pi r^2$ where r is the mean of r_1 and r_2, which are two independent radii of tumour mass.

Statistics: The data obtained from the experimental study were statistically evaluated by one way ANOVA using Graph pad-prism software. The experimental results were the mean ± SEM.

RESULTS AND DISCUSSION

The extract reduced the cancer cell number to $0.98 \pm 0.02 \times 10^6$ cells in the treated mice (Fig. 1). Also a decrease in tumour weight was noted in the CGA treated mice (Fig. 1). CGA administration increased the life span of ascites tumour harboring mice also (Fig. 1). Following inoculation with DLA cells, there was profound proliferation of tumour cells in the peritoneal cavity of the mice. As a result

the packed cell volume in the tumour control mice was found to be high (54.45 %). Intraperitoneal administration of the extract had reduced the packed cell volume to 36.7 % (Fig. 1). The effect shown by chloroform extract of *Gmelina asiatica* is similar to the effect produced by 5-flourouracil.

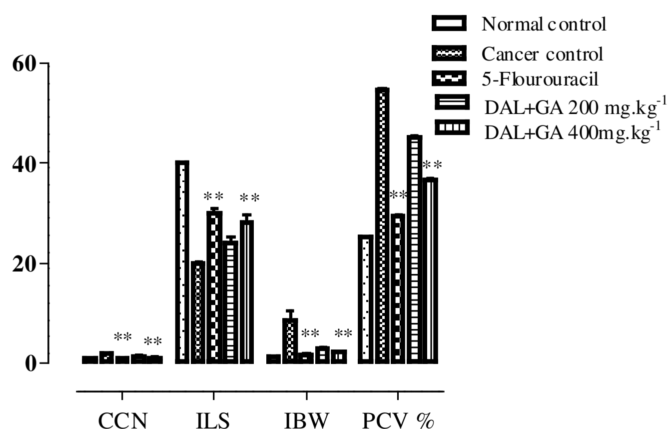

Fig. 1. Statistical analysis was carried out using one way ANOVA.Values are mean ± SEM of six animals in each group. **$p < 0.001$ compared to CCl_4 control

Regarding the effect of CGA on the hematological parameters, the tumour bearing mice showed an increase in the total WBC count and Packed cell volume but a reduction in the hemoglobin content of RBC. Also a reduction in platelet count when compared to tumor control (G2) was observed. At the same time, CGA (400 mg kg^{-1}) treatment brought back these parameters near to the positive control group treated with 5-fluorouracil (20 mg/kg) (Fig. 2).

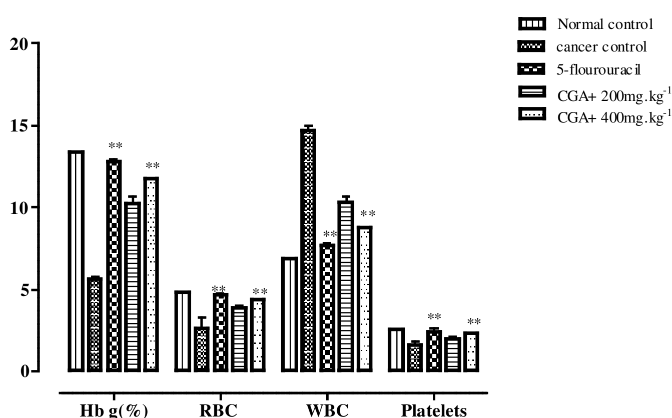

Fig. 2. Statistical analysis was carried out using one way ANOVA.Values are mean ± SEM of six animals in each group. **$p < 0.001$ compared to CCl_4 control

The inoculation of DLA cells caused significant increase in the level of total cholesterol, triglycerides, AST, ALT, ALP in serum when compared to normal group. The treatment of CGA at the dose of 400 mg kg^{-1} reversed the changes towards near normal level (Fig. 3). The treatment with standard 5-fluorouracil also gave similar results.

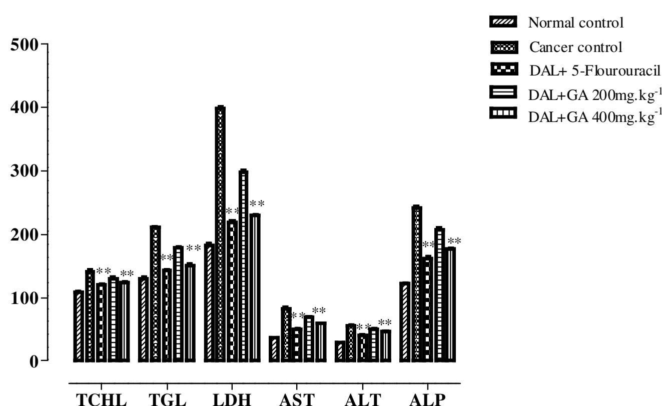

Fig. 3. Statistical analysis was carried out using one way ANOVA. Values are mean ± SEM of six animals in each group. $^{**}p < 0.001$ compared to CCl_4 control

There was also significant reduction in tumor volume of mice treated with GA (400 mg kg^{-1} orally). Tumour volume of control group was 12.05 ± 0.42 mL, whereas it was 7.18 ± 0.25 mL for the group treated with CGA 400 mg kg^{-1}, respectively (Fig. 4).

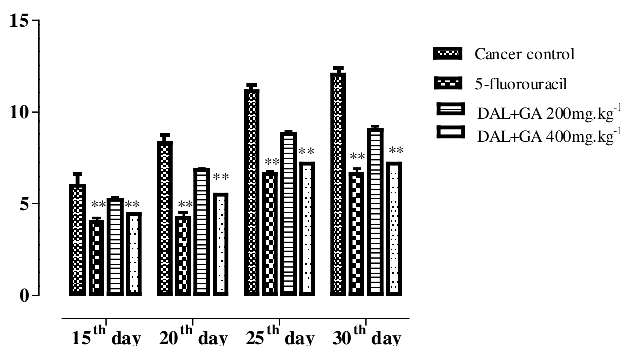

Fig. 4. Statistical analysis was carried out using one way ANOVA. Values are mean ± SEM of six animals in each group. $^{**}p < 0.001$ compared to CCl_4 control

Cancer is a group of more than 100 different diseases characterized by uncontrolled cellular growth, local tissue invasion and distant metastases[8] and the free radicals have been implicated in carcinogenesis[9]. The reliable criteria for judging

the value of anti-cancer drug is the prolongation of life span of animal. In this study, an increase in life span was also observed with CGA treated group. The DLA bearing mice administered with CGA at dose 400 mg kg^{-1} showed significant increase in average life span compared to the animals of tumor control group. In DLA tumour bearing mice, a regular rapid increase in ascitic tumour cell volume was observed[10]. The percentage increase in body weight, packed cell volume and number of viable tumour cells were found to be significantly less in CGA treated mice than the tumour control animals, indicating the anti-cancer nature of CGA.

The total WBC count was found to be increased, whereas RBC, platelets and Hb content decreased in cancer control when compared to normal control[11]. The reversal of Hb content, RBC, platelet and WBC by CGA treatment toward the value of normal group clearly indicate that CGA have a protective action on hematopoietic system.

Abnormal blood lipid profile has been associated with cancer. In Hodgkin lymphoma, high cholesterol level and low triglyceride level has been reported[12]. Abnormal liver function is observed in patients with *Hodgkin lymphoma*, so that these liver enzyme levels markedly increased in tumour bearing mice. ALT is an enzyme mainly derived from the liver, bones and in lesser amount from intestine, placenta, kidneys and leucocytes. An increase in ALP levels in the serum is frequently associated with the variety of disease. ALP comprises a group of enzyme that catalyzes the hydrolysis of phosphate esters is an alkaline environment, generating an organic radical and inorganic phosphate[13,14].

Markedly elevated serum ALP, hyperalkalinephosphatasemia, is seen predominantly with more specific disorder; including malignant biliary cirrohosis, hepatic lymphoma and sarcoidosis[15].

The significantly elevated level of total cholesterol, triglycerides, AST, ALT and ALP in serum of tumour inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversal of these changes towards the normal by CGA treatment indicates a protective effect on liver functions.

In the present study, the biochemical examination of DLA inoculated animal showed marked changes indicating the toxic effect of tumour. The normalization of these effect observed in the serum treated with CGA supported the potent anti-tumour activity of this compound. Regarding the effect of CGA on solid tumour volume, this study suggests the protective effect as evidenced by a decrease in the solid tumour volume. All this findings enable to conclude that CGA possess a protective effect against DAL.

Conclusion

The results of the present study has shown that chloroform extract of *Gmelina asiatica* (CGA) can significantly prolong the life span, reduce tumour volume and improve the haemotological parameters of the host. Further research work to is needed to establish the exact antitumour mechanism of action of *Gmelina asiatica*.

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