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Tijare RD

Dept. of Pharmacognosy and
Phytochemistry, T. John College
of Pharmacy, Bangalore - 83

Beknal AK

Dept. of Pharmacognosy and
Phytochemistry, T. John College
of Pharmacy, Bangalore - 83

Mahoorkar N

Dept. of Pharmacognosy and
Phytochemistry, T. John College
of Pharmacy, Bangalore - 83

In vivo anticancer potential of root tubers of *Asparagus gonoclados* Baker. against dalton ascitic lymphoma (DAL)

Tijare RD, Beknal AK, Mahoorkar N

Abstract

Herbals are valuable due to their medicinal properties and effectiveness in various diseases where allopathic drugs are less active such as psoriasis, hepatitis etc. In present study root tubers of the *Asparagus gonoclados* Baker were used as a drug for screening of the *in vivo* anticancer activity by using the DAL cell model.

The selected drug was evaluated for the pharmacognostical and phytochemical screening including antineoplastic activity. Pharmacognostical studies includes TS, maceration and powder drug analysis which gave comprehensive results which helps in proper identification. Drug had also under gone some standardization process which helps in development of some standards such as ash value (0.398% w/w), alcohol soluble extractives (41.09% w/w), water soluble extractives (31.26% w/w), moisture content and, Florescent analysis etc., for standardization

In present study selected drug has been screened for anticancer property by using DAL cells, where cancer was induced to all animals which are groped as normal, positive control, standard and some test groups. Study showed the comprehensive results for anticancer activity. Alcohol extract in high dose level showed the significant results in comparison to the disease group and normal with *p* value <0.001. Similarly aqueous extract showed the same response, henceforth, it gave a conclusion of dose dependant activity of the selected drug.

Keywords: *Asparagus gonoclados*, DAL cell model, root tubers of AG

Introduction

Nature is a wide source of the various medicinal plants, which has been distributed enormously on earth, which contains bushes, shrub, small plants, trees and aquatic plants. The detail investigated written information of different olden documents, by various researches, act as a source of the new comprehensive medicines with low side effects and high pharmacological properties. Presently many plants have been proved for various pharmacological properties such as anti-diabetes, hepatoprotective, antioxidants, anti-inflammatory etc., by different animal models. These all plants helps to fight against the complicated diseases effectively.

Among all complicated diseases cancer is one of the diseases, due to which many patients are suffering. Therefore, the herbal source act as an attempt to prove the same. In present study anticancer property of root tubers of *Asparagus gonoclados* Baker is proved by DAL cell model. *Asparagus gonoclados* is well known by the name 'Shatavari' in Ayurveda. Drug is a substitute for the *Asparagus racemosus* Wild, among 117 varieties worldwide, 17 are found in India¹. *Asparagus racemosus* is proved for various pharmacological properties such as immunomodulatory, antioxidant, hepatoprotective, antiulcer and anticancer, hence present study was performed to prove the anticancer potential of alcohol and aqueous extracts of the root tubers of *Asparagus gonoclados* Baker^[2].

Methods

In vivo anticancer activity was carried out by using Dalton ascitic lymphoma cells model. Study includes the screening of the drug for acute toxicity study and *in vivo* anticancer activity.

Acute toxicity study

OECD guidelines 425 (#26) method was followed to determine the therapeutic doses for the extracts of the study. No mortality of the animals was observed at the dose of 4000 mg/kg and hence 400 mg/kg (1/10th dose) and 200 mg/kg (1/20th dose) were used as the therapeutic doses

Correspondence:

Tijare RD

T. John College of Pharmacy,
Dept. of Pharmacognosy and
Phytochemistry, Kammanahalli,
Gottigere, Bannerghatta Road,
Bangalore-560083

for the alcohol and aqueous extracts of root tubers of the selected plant. No specific behavioural changes were noticed during 24 h in the animals subjected for acute toxicity studies.

Animals: Healthy and mature Swiss albino mice of both sexes weighing about 20-25g were acclimatized to the experimental conditions for about 2 weeks before subjecting them to experimental procedures.

Anticancer activity [3-5]

Inoculation of the DAL cells

For development of cancer in mice DAL cells were aspirated from the peritoneal cavity of cancer developed mice, which later mixed with phosphate buffer solution (PBS) of pH 7.2, centrifuged and the resulting pellet was washed with PBS. This process was repeated for 3 times. Different concentrations of the solution were prepared by mixing the cancer cells with PBS. Number of cancer cells of each dilution were then counted in WBC chamber of Neubauer slide. Dilution containing 10^6 cells/ml was prepared by trial and error method, which was then inoculated into the mice by intra-peritoneal injection.

The following formula was used for counting cancer cells.

$$\text{Cancer cell count} = \frac{\text{Number of cells} \times \text{Dilution}}{\text{Area} \times \text{Thickness of film}}$$

For evaluation of the anticancer activity the animals were divided in to 7 groups such as normal control, positive control, standard (treated with 5- fluorouracil, 20 mg/kg body weight by ip) of 8 animals each. Whereas group IV, V, VI and VII are labelled as alcohol extract (200mg/kg body weight), alcohol (400 mg/kg body weight), aqueous extract (200 mg/kg body weight) and aqueous (400 mg/kg body weight) respectively. To all above groups except normal and positive control treatment was given with respective drug for 14 days. Later different parameters such as tumour weight, number of cancer cells, packed cell volume as well as some haematological parameters such as WBC and RBC count were determined. Simultaneously among 8 mice, 3 animals from each group were under observation for checking life span of the animals.

Determination of packed cell volume

For determination of the packed cell volume, the mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube. The collected fluid is centrifuged at 1000 rpm for 5 min, to form a pallet like mass at the bottom of the centrifuge. Packed cell volume was determined by measuring the height of the accumulated mass of fluid at the bottom of centrifuge tube in terms of units.

Determination of tumour cell count

The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension is placed on the Neubauer counting chamber and number of cells in 64 small squares were counted.

Determination of life span of animals

Few of the animals in each group were separated and kept with proper supply of food and water for checking their survival time. The increase in survival is may be due to recovery from cancer or by modulation in immune system. The percentage

increase in life span (% ILS) was calculated from the following equation.

$$\text{Increase in life span} = (T - C \times 100) / C$$

Haematological studies

For determination of haematological studies blood samples were collected by retro-orbital puncture by using heparinized capillary tubes and used for RBC and WBC count.

Increased in tumour weight

Animals were consistently weighed at the specific intervals of 1 to 2 days throughout the study. Increase in body weight is indirectly related to the weight of the tumour.

Results and discussion

Cancer is a multi-mechanistic second largest disease in the world and requires a multidimensional approach for its treatment, control and prevention. Plant based drugs, forms an important component of total medicines available for treating various human diseases. The use of phytochemicals in cancer prevention has received considerable interest in the past few decades owing to certain discoveries with specific properties including anti-oxidant and anti-inflammation. Recently, a number of anti-cancer agents have become recognized therapies in the clinical settings which include: vinca alkaloids, taxols, podophyllotoxin, camptothecin and its derivatives [6] (Otsuki *et al.*, 2010). A number of additional plant-derived agents are currently under investigation for example Homoharringtonine, 4-Ipomeanol and β -lapachone [7] (Adriana *et al.*, 2001).

The plant '*Asparagus gonocladus*' is a well-known drug, proved as an alternate source of "Shatavari", which has been proved for many pharmacological properties, therefore, this was selected for screening of *in vivo* anticancer activity. The detail pharmacognostical studies of the selected drug showed the presence of root hairs, cortex cells, exo-dermis, cortical parenchyma, stellar portion in the centre, as well defined scattered vascular bundle with xylem and meta xylem. The different physico-chemical constants were determined for the process of standardization, such as total ash (0.398% w/w), acid insoluble ash (1.033% w/w), water soluble ash (1.40% w/w), alcohol extractives (41.09% w/w) and water soluble extractives (31.26% w/w) etc. Fluorescence analysis of the drug powder showed the various behavioral changes with the short and long wave length.

Before screening the drug for pharmacological activity, acute toxicity study of the plant drug was carried out which showed nontoxic nature up to 4000 mg/kg body weight by following the OECD guidelines. For development of tumour, 10^6 cells/ml was inoculated to induce tumor.

Myelosuppression and anaemia have been frequently observed in ascites carcinoma condition. Similar findings were observed in our present study. In DAL bearing tumour control animals, elevated total WBC count and reduced haemoglobin content was observed. Drug extracts showed a protective effect on hematopoietic system by reversal of total WBC cells and haemoglobin content in DAL bearing animals towards the value of normal group animals when compare to DAL bearing ascitic tumour animals.

We observed significant inhibition of solid tumour volume and reduction of body weight in solid tumour bearing animals when compared to control DAL induced solid tumour animals which suggests that the inhibitory effect of *A. gonocladus* is

systemic, not only related to its local cytotoxic effect. This inhibitory effect on tumour volume and protection of hematopoietic system was comparable with the result produced by the standard drug 5- fluorouracil.

The major non-protein thiol, GSH is required for the tumour cell proliferation and its metabolism^[8] (Guruvayoorappan and Kuttan, 2007). Cancer cells have higher GSH levels than the surrounding normal cells, which is characteristic of higher cell proliferation rate and resistance to chemotherapy. Scientific evidence showed that combining GSH depletion using 1, 3-Bis (2- chloroethyl)-1-nitrosourea chemotherapy with superoxide dismutase gene therapy could be considerably successful in the treatment of breast cancer. When the intracellular GSH levels are low, the cells are more susceptible to ROS attacks. Increased ROS might activate different intracellular oncogenic pathways which lead to activation of tumour genesis process^[9] (Weydert *et al.*, 2008). However, the excessive levels of ROS stress can also be toxic to the cancer cells. Therefore, changing ROS levels by GSH modulation is a way to selectively kill cancer cells without causing toxicity to normal cells^[10] (Makiya, 2008; Trachootham *et al.*, 2009). Administration of *A. gonoclados* Baker extracts significantly reduced the level of intracellular GSH in extract treated DAL tumour cells when compared to the non-extract treated DAL bearing animals. Moreover, the treatment with extract also reduced the level of Nitric oxide production in serum and tumour cells when compared with tumour control animals. Nitric oxide is an important regulator

of tumour growth and is involved in various pathophysiological process including inflammation and carcinogenesis^[11] (Hong, 2002). Study showed the significant increase in life span of the animals in comparison to positive control, similarly significant decrease in packed cell volume and cancer cell count with p value > 1000, which is shown in Fig. 1 and 2 and Table no 01. The positive response of the drug may be due to the presence of the various phytoconstituents in plant. It may be due to the combined effect of all phytoconstituents towards the cancer cell death. It has been observed that different phytoconstituents such as β -sitosterol also showed anti-oxidant and anti-inflammatory activity and is used in the treatment of inflammatory disorders, breast cancer and colon cancer^[12] (Padmasri and Sarada, 2011). Phenolic and flavonoids display a wide range of biological and pharmacological properties and normally scavenge the free radicals and play an essential role in prevention and therapy of cancer. It helps to conclude that different phytoconstituents and phyto-pharmaceuticals, individually act against cancer.

Drug in higher dose showed the significant *in vivo* anticancer activity, which may be due to the presence of different plant constituents.

Conclusion

Present study proved the anticancer potential of the *Asparagus gonoclados* Baker, which will be a source of new drug for the treatment of cancer with less normal cell toxicity.

Table 1: *In vivo* anticancer activity of *A. gonoclados* Baker

Groups	WBC(cells/ μ L) $\times 10^3$	RBC (millions cell/ micro lit	PCV (%)	ILS (%)
Normal	5.46 \pm 624.82	5.28 \pm 0.037	-	-
+Ve control	12.62 \pm 255.73	2.37 \pm 2.37	27.4 \pm 0.871	20 \pm 1.12
Standard	5.66 \pm 227.16	4.94 \pm 0.09	9.6 \pm 1.34	81 \pm 4.1
AG.Alc. 200	9.70 \pm 70.11	3.48 \pm 0.11	21 \pm 1.0	24 \pm 1.87
AG.Alc.400	6.02 \pm 101.98	4.84 \pm 0.05	10.6 \pm 1.288	60 \pm 4.18
AG. Aq.200	9.20 \pm 398.75	3.3 \pm 0.08	19 \pm 1.225	20 \pm 3.873
AG.Aq.400	6.40 \pm 170.29	4.8 \pm 0.12	9 \pm 1.14	50 \pm 3.536

Results are determined by comparing with positive control. Values are expressed as Mean \pm SEM. ANOVA, P value for all concentration of alcohol and aqueous extract are 0.001.

*** $p < 0.001$, Tukey Kramer.

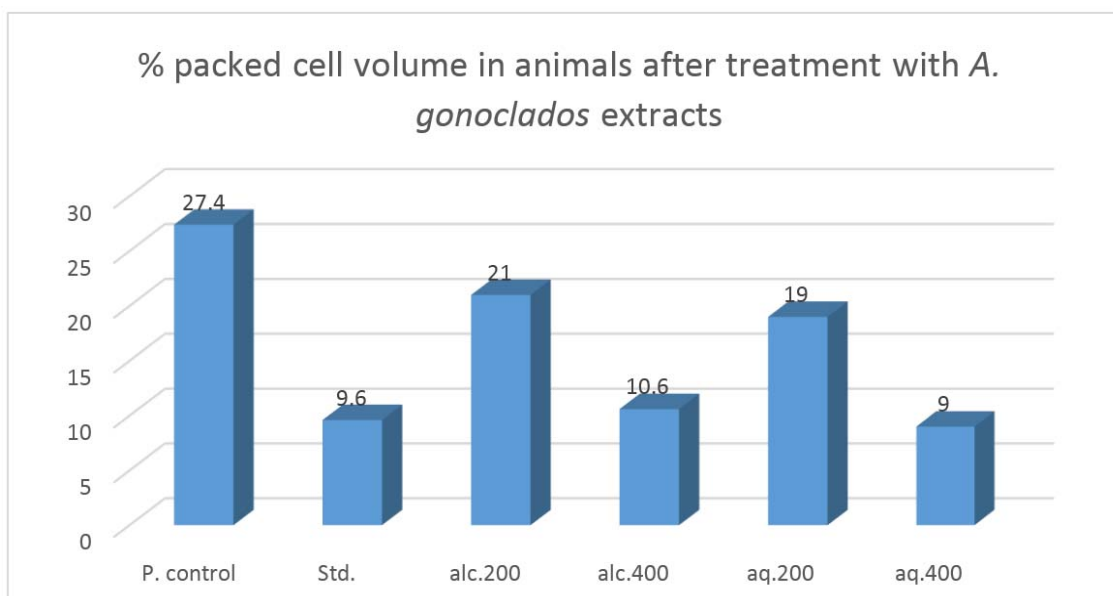


Fig 1: Effect of *A. gonoclados* Baker on packed cell volume in treated animals

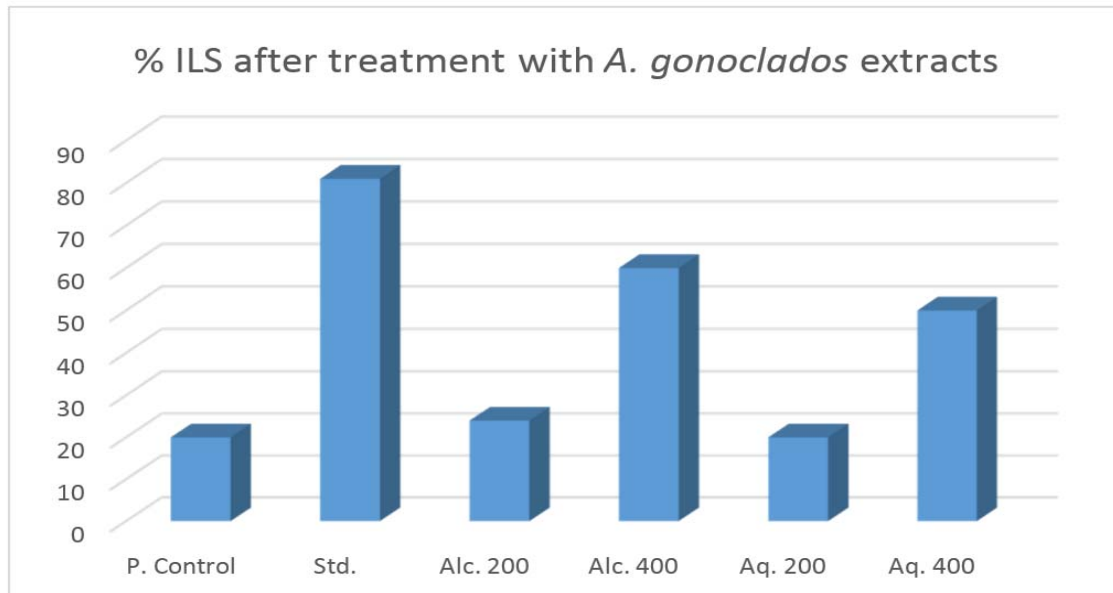


Fig 2: Increase in % Life span on treatment with different drugs and extracts of *A. gonoclados* Baker

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