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Screening the Anti-Cancerous Efficacy of *Achyranthes aspera* Linn. using Animal Model Swiss Albino Mice

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Abstract:

Achyranthes aspera is a one of the important traditional medicinal plant. In the present investigation anticancer efficiency of *A. aspera* was evaluated in Swiss albino mice after treated with mineral oil. In Swiss albino mice the cancer state was induced by intraperitonial injection of mineral oil at a dose of 1 ml/kg of body weight for 21 days. The tail length of the normal mice was 9.6 cm whereas the mice with metastasize tumor in the head had a tail length of 5.8 cm, metastasize throat cancer mice had 5.9 cm of tail length and the mice with plasmacytoma alone had the tail length of 5.4 cm. The anticancerous activity of *A. aspera* leaves was tested against mineral oil induced cancer mice. Simultaneously a group of mice was first intraperitoneally injected with the sublethal doses (3 mg/ ml and 1.5 mg/ ml) of ether extract for 15 days. After 15 days the extract given mice were treated with 1 ml/kg of mineral oil periodically for 21 days. It was found that none of the mice got the symptoms of cancer. The present work clearly indicates that the ether extract at the concentration of 3 mg/ ml is very effective in reducing the cancer symptoms.

Keywords: *Anti-Cancer activity, mice, mineral oil, ether extract* and *Achyranthes aspera.*

Introduction:

Traditional systems of medicine, Ayurveda were the basis of the health care systems in India until early years of twentieth century. Ever increasing costs of treatments and growing knowledge of relationship between diet and disease have attracted the health researchers towards traditional herbal medicines and prospection of plants for new drugs (Anil Kumar Dhiman, 2006). New kinds of vaccines and technologies are available to prevent epidemics of infectious diseases. However, the therapeutics base biochemical on small molecular weight that mimicker the body's lost function will remain the most important component of evolving health care systems. Knowledge based on Ayurveda is a product of many centuries of actual experience. This knowledge can be converted into new effective non-traditional formulations (Chatterjea and Rana shinde, 2005). In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care (Ramkrishna, 2003). According to an estimation of world health organization (WHO), approximately 80% of people in developing countries rely on traditional medicines for primary health care needs (Anil kumar Dhiman, 2006). The presumption of world health organization (WHO) is that the edibles, particularly of plant derived, have medicinal values. This is the reason why, besides the nutritional values of plant edibles, medicinal values have been searched going through modern literature (Basak, 2000).

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material (Brindha *et al.,* 2008). This fact is well documented in the inventory of medicinal plants, listing over 20,000 species. In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, large segments of the world population

still like drugs from plants. Hence, in the present investigation to check the anti-cancerous activity of different organic solvent extracts of *A. aspera* leaves was carried out.

Materials and Methods:

Preparation of extracts: The plant extracts were prepared in hot extraction method.

Hot method: Ten gram of powdered plant material was taken in clean sterile soxhlet apparatus and extracted with 100 ml of different solvents (low polar to high polar) like as ether, hexane, butanol, chloroform and water. After extraction the extracts were dried in room temperature until extract reach into solid form. From the crystal /solid extract made into different concentration for analyse LD_{50} using mice, from LD_{50} concentration find out the sub lethal concentrations for further analysis.

Animals and treatment: Swiss albino mice of either sex were used in the present study. The animals were housed in polypropylene cages and maintained at 27ºC ± 2ºC, relative humidity 60± 5% and 12hrs light /dark cycles. They were fed with standard diet, water and pellet feed during the experimental period. For the experimental study, mice weighing 24± 0.2 gm (35 days old) were recruited from the acclimatized stock. Mice were grouped into five different groups (normal, mineral oil exposed, leaf extract exposed, mineral oil + Leaf extract exposed and mineral oil treated cancer mice exposed to ether extracts) with six individuals each. Different leaf extracts (ether, hexane, butanol, chloroform and water) were screened for anti cancerous activity using cancer mice. Before performing the experiment, ethical clearance was obtained from institutional animal ethics committee. One tenth (1/10) and one twentieth (1/20) of lethal doses of the extracts were given to the animals for the evaluation of anti cancerous activity.

Group 1: ***Control:*** The grouped mice were maintained in regulated light and temperature conditioned rooms without any treatment.

Group 2: ***Toxicity analysis in mice treated with mineral oil:*** Six mice were injected intraperitoneally with 1 ml/Kg of mineral oil. The intraperitoneal injections were given periodically for 21 days.

Group 3: ***Toxicity analysis in mice exposed to ether extract:*** A test substance, extracts were given to animals intraperitoneally every day at the dose level of 250, 500 and 1000 mg/ 10 ml. All animals were observed for toxic symptoms and mortality for 96hrs, from the mortality rate LD50 value (300 mg/ml) was calculated. The sub lethal dose was found to be 1/10th and $1/20^{th}$ concentration of 96hrs LD_{50} doses. The sub lethal concentration was 3 mg/ml and 1.5 mg/ml for extracts administered to a group of mice and used as extract control.

Group 4: ***Treating the cancer mice with extracts:*** The mouse with the metatasize tumor in the head, throat and plasmacytoma were treated with extracts for 15 days.

Group 5: ***Treating mice with extracts and mineral oil:*** Thus the sub lethal doses (3 mg/ml and 1.5 mg/ml) of ether extracts were given intraperitoneally to corresponding mice. The doses were given periodically to the mice for 15 days and kept in the same temperature and feed as that of the control mice. After 15 days the mice were intraperitoneally injected with 1 ml/Kg of mineral oil for 21 days.

Study of cancer symptoms: The cancer symptoms were studied by observing the length of tail, swelling uncontrollable growth in body parts and enumeration of T cell counts.

T cell count: The T cell count for the normal mice, extracts administered mice and the cancer mice were compared. 1.5 ml of blood from normal mice and other treated mice were collected in a heparin pretreated vials. It was introduced into the sterile beaker containing 3 glass beads. It was then continuously stirred till no sounds were heard from the beads.

Table 1: Comparison of tail length of mice before and after treating with mineral oil.

Types of mice	Tail length in cm	
	Before treating with mineral oil	After treating with mineral oil
Normal mice	9.6cm	9.6cm
Mouse with plasmacytomas alone	9.7cm	5.4cm
Mouse with metastasize tumor in the head	9.6	5.8cm
Mouse with metastasize tumor in the throat	9.5cm	5.9cm

This de-fibrinated blood was taken and diluted with equal volume of physiological saline. 1ml of lymphopreb solution was taken in a vial to which added the diluted blood in slanting position using pipette. The content of the vial was centrifuged at 1400 rpm for 20 minutes. The interphase was removed using pipette. The cells were centrifuged at 1400 rpm for 20 minutes and the pellets were washed with 1ml saline. The pellet was resuspended in 100 µl of RPMI 1640 medium.

12-14 cm of drinking straw was cut. One end of the straw was slantly cut and sealed by slightly heating the tip in a flame. Nylon wool fibres were finely teased using a pair of forceps and the teased fibres were packed (loosely) into the straw. Washed the straw using 5ml of physiological saline and made a small opening at the sealed end of the straw to drain the saline. It was then filled with 3ml of RPMI1640 medium in a horizontal position. The column was now kept in incubator at 37ºC for 30 minutes in horizontal position. Resuspended lymphocytes were loaded into the activated nylon wool column. Then the column was held vertically above an eppendorf tube, now hot saline (about 60ºC) was added. The hot saline passing out of the column was collected. To this 0.2 ml of 1% SRBC (Sheep Red Blood Corpuscles) was added and the mixture was centrifuged for 12 minutes at 1600 rpm. After centrifugation the samples were incubated in the refrigerator at 4ºC for 5 minutes. The pellet was resuspended in the 100 µl of RPMI medium. Then a drop of it was taken in a clean dry slide, observed and enumerated T cells under the microscope (20x/40x) for rosettes. Number of T cell rosettes formed were observed among 100 lymphocytes and tabulated.

Results and discussion:

Anti cancer study

The anticancerous activity of *A. aspera extracts were* checked with the cancer mice. The present results show that ether extract of the plant leaves has efficient anticancerous activity compared to other extracts. The anticancerous activity, phytochemical screening of *A. aspera* and its impact on cancer cells was analyzed and recorded.

Reduction in the tail length of cancer mice

Reduction in the tail length of the mineral oil induced mice confirms the presence of cancer in those mice. The tail length of the normal mice was 9.6 cm whereas the mice with metastasize tumor in the head had tail length of 5.8 cm, the metastasize throat cancer mice had 5.9 cm of tail length and the mice with plasmacytoma alone had the tail length of 5.4 cm. The comparison of the tail length of mice before and after the mineral oil injections was shown in the Table 1.

T cell count

The increase in T cell count further confirms the presence of cancer in the mice. The comparison of T cell count for normal and cancer mice before and after giving extracts is given in the Table 2.

The anticancerous activity of *A. aspera* leaves was tested against mineral oil induced swiss albino mice (group V). It was found that none of the mice got the symptoms of cancer. This result is in agreement with the earlier reports of Careonal, (2000). Careonal, (2000) stated that the *A. aspera* leaves contain Vitamin

Table 2. Comparison of T cell count for normal mouse and cancer mouse before and after giving extracts.

Types of mouse	Tcell count before giving extracts	Tcell count after giving extracts
Normal mouse	62/100 lymphocytes	62/100 lymphocytes
Mouse with plasmacytoma and metastasize tumor in the head	96/100 lymphocytes	81/100 lymphocytes
Mouse plasmacytoma alone	84/100 lymphocytes	83/100 lymphocytes
Mouse with plasmacytoma and metastasize tumor in the throat	93/100 lymphocytes	94/100 lymphocytes

C and beta-carotene that has the property of anti-tumoral activity. Similar findings were obtained in *Mallotus peltatus* leaf extracts administered animals (Bhuttacharya *et al*., 2002), from this phytochemical obtained in plants were used as an anti cancerous agent.

The group II mice developed plasmacytoma, among the six, one of the mice was found to develop metastasize tumor in the head and one got metastasize tumor in the throat. The mouse with the metatasize tumor in the head, throat and plasmacytoma were treated with ether extract for 15 days. There was reduction in the cancer symptoms for the ether extract treated cancer mouse.

The present work clearly indicates that the ether extract at the concentration of 3 mg/ml is very effective in reducing the cancer symptoms. These results are in agreement with the earlier reports of Careonal, (2000) and Bhuttacharya *et al*; (2002). Because of the easily soluble nature of this plant in organic solvents and of its anticancerous activity, suggests that *A. aspera* leaves can be used in the anticancerous drug preparation in alternative medicine. This suggests that using the *A. aspera* leaves is cheap and the economic herbal drugs can be prepared for cancer treatment. The use of *A. aspera* leaves was highly promising for cancer treatment.

A. aspera leaves were traditionally used by the tribal people for the treatment of tumors (Joy, 1998). Hence in the present study different extracts of *A. aspera* leaves were tested with the cancer mice. The treatment showed that the ether extracts of *A. aspera* leaves has the greater anti cancerous activity and it is highly promising for the further phytochemical evaluation and this will be continued in the further studies.

The free radicles present in the mineral oil have the capability to bind covalently with the DNA and interact with purine; pyrimidines and phosphodiester groups of DNA. Thus the normal cells turn to cancerous cells. If the cancer cell spread throughout the body along with tumor site, that type of cancer is malignant tumor. *A. aspera* leaves has been used traditionally by tribal people for the treatment of inflammation, swellings, wounds and tumors. But hitherto, no work has been carried out scientifically to prove the efficacy of this plant to inhibit the growth of cancer cells. Hence, in the present study mineral oil induced swiss albino mice with plasmacytoma and metastasized plasmacytoma with head and throat tumors were chosen to find out the anticancerous properties of *A. aspera* leaves. The antioxidant property of *Achyranthes aspera Linn* leaves prevents the damage caused by the carcinogens to the DNA that disturbs the cell function (Murthy and Aleyamma Mathew, 2004). Hence, the present study promotes the utilization of plant-derived substances to prevent the growth of cancer cells.

Inspite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects (Jayakar *et al.,* 2003). Therefore, a systematic approach should be made to find out the efficacy of plants against cancer. Hence, the present study strongly suggests that the utilization of plant decoction to prevent the growth of cancer cells and inflammatory disorders. Further studies involving the purification of the chemical constituents of the plant and the investigation in the bio chemical pathways may result in the development of a potent anti-cancerous agent with better therapeutical index.

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