3.3.2 Number of books and chapters in edited volumes/books published and papers published in national/ international conference proceedings per teacher during last five year

Sl. No.	Name of the teacher	Title of the book/chapters published	Title of the paper	Title of the proceedings of the conference	Name of the conference	National / International	Calendar Year of publication	ISBN number of the proceeding	Affiliating Institute at the time of publication	Name of the publisher
1	Neelu Lamba	Crime in Society/Crime against Older Adults					2022	978-93-81778-14-2	IIS University, Jaipur	Associated Publishing House, Agra
2	Neelu Lamba	Research Methodology/Academic Research					2021	978-93-81778-92-0	IIS University, Jaipur	Associated Publishing House, Agra
3	Manisha Sharma	Roll of BioTechnology in Medicinal And Aromatic Plants/Cancer: Hornest Nest of Medical Sciences					2020	8188279390	Rajasthan University	Ukaaz Publication, Hyderabad
4	Manisha Sharma	Roll of BioTechnology in Medicinal And Aromatic Plants/In Vitro Perspectives of the Healing Herbs Cuminum Cyminum And Trachyspermum ammi.					2020	8188279390	Rajasthan University	Ukaaz Publication, Hyderabad

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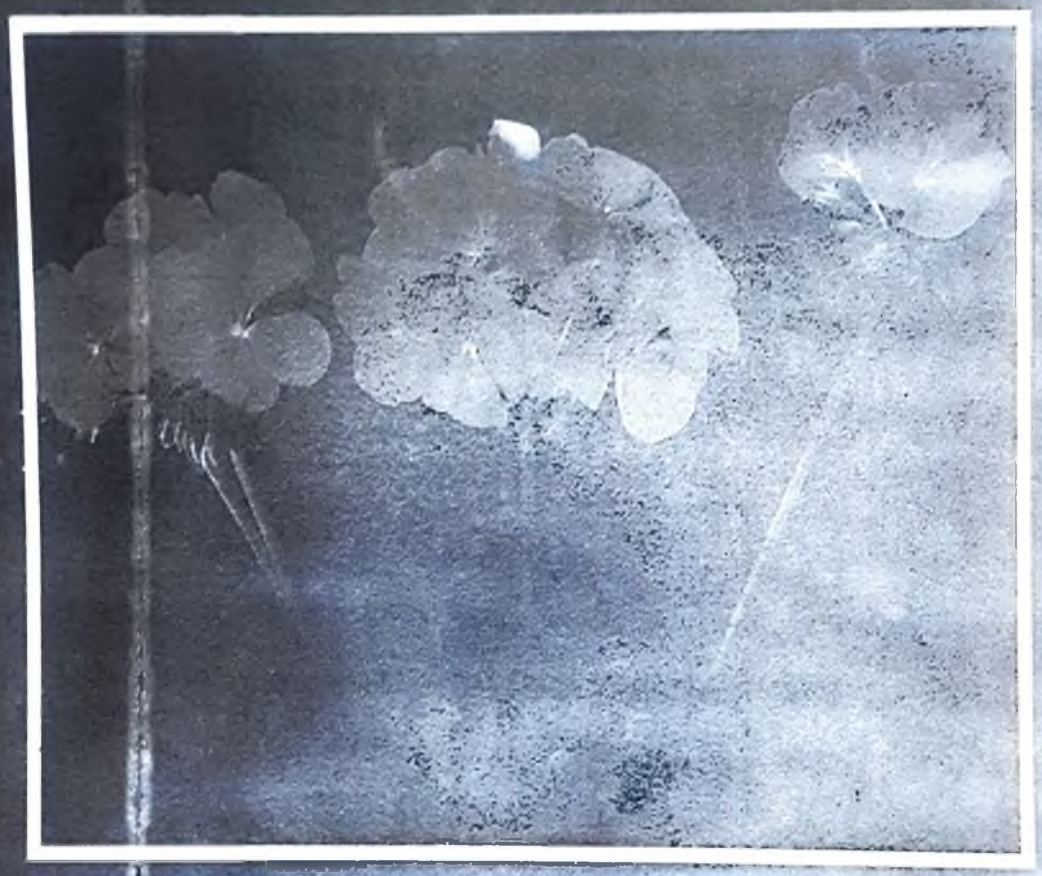

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VOLUME-IV : CONTENTS

IN VITRO PERSPECTIVES OF THE HEALING HERBS *CUMINUM CYMINUM* AND *ACHYSPERMUM AMMI*

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Chapter outline

1. INTRODUCTION :

The foundation of life was laid with the emergence of plants as the living in habitants during the volcanic age. Plants further enabled the advent of present life forms by altering the O_2 level in the atmosphere. Sustainment of these forms was ensured by the basic food products supplied by the plants. Thus, plants form the life line of the spectrum of fauna residing our mother earth. This harmonious interaction between the flora and fauna could be well enunciated by a tamil proverb which goes as follows:

"No fodder, no cattle; no cattle, no manure; no manure, no crop"

Besides providing the basic amenities of life to man, nature also keeps in her folds luxuriant herbal cover in deep ravines, nooks, corners, rock ledges *etc.* which possess medicinal properties. This also confirms on older saying 'For every disease that arises on this planet, plants or herbs give a cure" (Karnick and Pathak, 1983).

It is thus clear that primitive human societies largely depended on natural forest wealth for food, medicine, shelter *etc* (Gunther, 1945; Tarafdar, 1986; Laxman and Narayanan, 1988; Nazaruddin *et al.*, 1996). In fact, all the initial knowledge about the beneficial and curative properties of plants was the outcome of early human's quest to find herbs useful for satisfying his thirst, hunger and for the treatment of his injuries or illness (Pandey, 1989; Jain, 1994).

The tribal people and aborigines all over the world used and still use an enormous range of wild plants for their basic needs, sustenance and livelihood. There exists a direct relationship between these people and plants; study of such a relationship has been termed as 'Ethnobotany' (Maheshwari, 1983).

It is thus obvious that ethnobotany is the study dealing with all the aspects and relationships between man and plant resources. This makes it an interdisciplinary science drawn from different aspects of anthropology, botany, archaeology, ecology, economics and medicine.

Right from the ancient times people in India have been using herbal medicines, this is well accounted even in historic scriptures. Even today, years after the introduction of modern medicine in our country, the Indian system of folk medicine continues to provide medicinal relief to nearly 80% of our rural people. Remedy through herbs is a unique but genuine and

scientific healing system. Simplicity is one of the most reasonable features of this system of treatment with very little danger of any side effects where patient is cured in a smooth and natural way (Saxena and Tripathi, 1989).

The medicinal properties of these plant parts are due to the presence of certain chemicals with some definite physiological action on the human body system. Hence, in recent years the significance and awareness about medicinal plants has increased beyond mere anthropological curiosity (Bickmann, 1984).

It has been a well known fact that all systems of traditional Indian medicine have their roots in one way or the other in folk and household remedies. In addition, many modern drugs are derived from natural plant products or are chemical simulations of such substances. Therefore, the present times are witnessing revival of traditional drugs and knowledge of the medicinal plants.

However, the dictum – "Necessity is the mother of invention" fully applies to the circumstances of rural or primitive societies, which have to discover solutions to almost all their needs and problems from the natural resources around them. The plants used by them for a variety of purposes may not always be the most suited but they are the best available locally. The study of indigenous home remedies can thus serve to validate and enhance existing local uses and to provide clues to remedies having world wide potential.

Two such locally available herbs constituting an integral part of our diet, possessing healing potentiality to cure different ailments are

- ***Cuminum cyminum***
- ***Trachyspermum ammi***

Their distribution, taxonomic description and medicinal properties have been mentioned below:

2. CUMINUM CYMINUM :

2.1. Distribution : *Cuminum cyminum*, commonly known as cumin or Jeera belongs to family Apiaceae. It is a native of upper Egypt, Turkestan and the Eastern Mediterranean, where it has been used since the earliest times. It is grown chiefly in Iran, India, Morocco, China, Southern Russia, Indonesia, Japan and Turkey. Iran is major exporter of 'green cumin' (Pruthi, 1976), where it is mainly cultivated in the Khurasan province. In India, it is cultivated in almost all states (except Assam, Kerala and West Bengal) and it also stands among chief exporters. During past four-five years Iranian and Indian cumin is continuously being popularised among other countries. The chief producers of cumin in India are Uttar Pradesh, Rajasthan, Gujarat and Tamil Nadu. Growing regions for cumin in Rajasthan are Jaipur, Jodhpur, Pali, Jalore, Barmer, *etc.*

The dried fruit of the plant (*i.e.* cumin seed) is a well known condiment and spice. The physico chemical composition of the seed is, moisture 6.2%, carbohydrates 35.5%, protein 17.7%, fat 23.8%, crude fibre 9.1%, mineral matter 7.7%, Ca 0.9%, P 0.45%, Fe 0.48%, Na 0.16%, K 2.1% and vitamin B (vit B_1, B_2, Niacin), C and A. The seed contains fixed oil which is greenish brown with strong aromatic flavour (10%) as well as volatile oil (2 to 4%). The volatile oil gives a strong distinctive pleasant odour with a warm and somewhat bitter taste. The chief content of volatile oil is cuminaldehyde (20-40%). Cumin seed is an important ingredient used for flavouring soups, pickles, seasoning breads and cakes. In India, the seeds have long been employed as a stimulant, carminative and stomachic. Besides, it provides astringent and cooling effect. It is used in veterinary medicine also. Cumin is also reported to posses antifertility activity (Kant *et al.*, 1989). The essential oil has been reported to possess

antimicrobial activity (Menghini *et al*., 1987). The oil is also employed for manufacturing liquors and cordials. Residue left after volatile oil extraction is used in soaps and fat industries.

2.2. **Taxonomy** : Cumin is a small, low growing, annual, aromatic herb, height of the plant reaches to 0.3m, the plants have sparingly branched tap root system, stem in much branched and angular, leaves are exstipulate, alternate with lamina deep green and highly dissected (decompound), bearing elasping (sheathing) base. Inflorescence appears about 60-66 days after germination. Flowers are borne terminally on stems. They are white coloured and arranged in compound umbels. Each umbellet has involucre of small bracts and consists of six florets of 3-5 form fruits. Each floret is pedicellate, zygomorphic due to unequal length of sepals, often bisexual and epigynous. Androecium comprises of 5 polyandrous antisepalous stamens bearing long filaments and dithecious anthers. Gynoecium bicarpellary, syncorpous, ovary inferior, bilocular, one pendulous ovule in each locule in axile placenta and is terminated by two styles and stigmas on the stylopodium. Fruit is schizocarpic cremocarp, having hairy ridges, particularly the secondary ridges; 6 vittae or oil tubes, strong heavy odour. Each mericarp contains a single seed.

2.3. **Medicinal uses :Healing power and curative properties :** The fruit is a rich source of thymol. Thymol is used as an anthelmintic against hookwarm infections and also as an antiseptic in many proporietary preparations. It is a stimulant, which increases the secretion and discharge of urine and relieves flatulence. It strengthens the functions of stomach and arrests any bleeding.

Digestive disorders : Cumin seeds are very useful in digestive disorders like biliousness, morning sickness, indigestion, atonic dyspepsia, diarrhea, malabrorption syndrome, and flatulent colic. One teaspoon of cumin sees is boiled in a glass of water and the decoction mixed with one teaspoon of fresh coriander leaf juice and a pinch of salt. This decoction can be taken twice daily after meal as a medicine for diarrhea.

Constipation : Cumin fruits are very useful in constipation One teaspoon of cumin fruit and one teaspoon of khirni are crushed, ground and mixed with half cup water. The patient is allowed to drink it. This decoction can be taken once a day only. Half teaspoon of mustard seeds and half teaspoon of cumin seeds are ground together and the mixture is taken with water. It can be taken once a day, till the symptoms disappear.

Cholera : Cumin powder mixed with mishri and curd is useful in cholera. Half a teaspoonful cumin powder and one teaspoon mishri are mixed in half cup curd. Thereafter, it is given to the patient to eat once daily.

Insomnia : Cumin is valuable in relieving sleeplessness. A teaspoon of the fried powder of cumin seeds mixed with the pulp of a ripe banana can be taken at night to induce sleep.

Renal colic : Black cumin seeds mixed with caraway seeds and black salt is useful in renal colic. About 20 gms of cumin seeds, 12 gms of caraway seeds and 6 gms of black salt are ground together and mixed with a little vinegar. This mixture can be taken in doses of 3 gms every hour till relief is obtained.

Common cold : Dilute cumin water is an antiseptic beverage and very useful in common cold and fevers. To prepare cumin water, a teaspoon of cumin is added to boiling water, which is allowed to simmer for a few seconds and set aside to cool. If the cold is associated with sore throat, a few small pieces of dry ginger should be added to the water. It soothnes throat irritation.

Secretion of breast milk : A decoction of cumin seeds mixed with milk and honey, taken once daily during the entire period of pregnancy, helps the healthy development of the foetus, eases child-birth and increases the secretion of breast milk.

Rheumatism : The oil extracted from the seeds is beneficial in the treatment of rheumatic and neuralgic pain.

Earache :About half a teaspoon of the seeds is heated in 30 ml of milk till the essence of the seeds permeate the milk. The milk is then filtered and used as ear drops.

Scorpion bite:One teaspoon Ajowain is ground mixed with water and put over the affected area.

Fever with abdominal disorder : 2 gms Ajowain, one cup seeds of bajra and 2 gms leaves of chircheta are boiled in water and strained. This is given to the patient to drink. It is to be given three times a day for three days.

Gastric problem : One teaspoon ajowain, one teaspoon harad and 200 gms flesh of leaves of gwarpatha are mixed together and dried in shade. It is then crushed, ground and made into powder one teaspoon of the powder is given every day till the symptoms disappear.

Loss of appetite : Ajowain is used as appetiser. One teaspoon ajowain is to be immersed in the cold water overnight in an earthen pot. It is to be taken morning and evening, twice daily for one month.

Menstruation problems : One teaspoon ajowain is crushed, ground mixed with one table-spoonful *ghee* and *bura* which is given to the patient to eat. It is to be given for three days.

Chest pain : One teaspoon of ajowain is taken in a cup of water and boiled till the volume reduces to half cup. Two tablespoon jaggery is added to it and mixed well.

Other uses : Aphrodisiac: Ajowain seeds combined with the kernel of tamarind seeds are an effective aphrodisiac. These should be fried in equal quantity of pure ghee, powdered and one teaspoon of this powder, mixed with a tablespoon of honey, taken daily with milk before retiring, makes an excellent aphrodisiac.

The greyish brown fruits or seeds are used as a spice, in flavouring numerous foods, as antioxidants, preservatives and in medicine.

Although, the above mentioned crops are easily available and grow in abundance, however, owing to their economic and medicinal value they emerge as potent candidate crops to be investigated *in vitro*.

Over the years considerable progress has been made in scientific field and tissue culture methodology is increasingly being applied for propagation of crops as a supplement to the conventional method. Whereas classical methods take long to introduce the desirable traits in the crop, the technique of plant tissue culture is decidedly superior which is now being engaged in a number of industries like agriculture, horticulture, silviculture, pharmaceutics *etc.* Besides production of a large number of plants in considerably lesser time, yet another desirable practical utility of plant propagation *in vitro* is in obtaining disease free plants. Besides this cell and tissue culture techniques offer ample scope of generating desired heritable variations in the regenerants (Batra, 1995). Above all cell cultures provide simple system which is more convenient in handling as compared to field grown plants.

These beneficial factors substantiate the use of modern techniques like tissue culture for obtaining quantitatively and qualitatively superior plants. Therefore, the tools of plant cell culture are increasingly being applied to a wide range of biotechnology ventures and in particular to the clonal propagation and genetic improvement of crop plants. For this the approaches and methodologies must be specifically adopted to the differing problems and potentialities of each crop and to the varying responses of plants.

The following text presents a summarised account of the experimentation conducted in the two candidate crops in the authors' lab. These experiments ultimately led to the establishment

of efficient, reliable and rapid regeneration protocols. These studies may thus be considered as a remarkable step towards further advanced research in the sense that application of biotechniques in a plant system requires a complete and quick plant regeneration system.

For all the *in vitro* experiments performed on both the crops, the explants (starting material) were taken from two weeks old aseptically grown seedlings on paper bridges with distilled water or half strength MS (Murashige and Skoog, 1962) medium [Plate 1; Fig. A-D]. MS medium supplemented with hormones was invariably used for maintaining all the cultures. The aseptic manipulations were conducted in the laminar air flow bench pre-sterilized with ultraviolet light for forty minutes. The cultures after inoculation were kept in a growth chamber under controlled conditions of light and humidity.

Outline, observation and results of the undertaken experiments are narrated below:
Micropropagation (production of plantlets *in vitro*) has emerged from theoritical confines to establish itself as a multidollar industry. The intent of micropropagation is an increased production of plants that are uniform and predictable of selected qualities. However, these procedures should strive for optima, with respect to certain parameters, namely the initial explant characteristics, nutrient formulation and culture environment.

Micropropagation is usually achieved in three steps

(1) Establishment of explant
(2) It's multiplication and
(3) Then it's rooting, which is a lengthy and time consuming process.

However, in cumin as well as ajowain, short and quick system for complete plant regeneration has been established using shoot tip as explant.

In case of *C. cyminum* production of regenerants via apical bud culture proved quite feasible. Main advantage of culturing these buds over any other mode of plantlet production lies in the fact that they harbour preexisting meristem. Hence these could be triggered easily for shoot bud proliferation. The experiment initiated with the inoculation of shoot tips on IAA containing MS basal medium. Although, multiple shoot induction could not be elicited, however, inherent meristematic property of the explant was considerably enhanced. Thus, an increase in length was observed by the first week of inoculation, with the emergence of new shoots from the meristematic cells present in the explant [Plate 2; Fig. A]. By the second week the shoot development was vigorous. With the development of shoots, rooting also concurred just simultaneously proportionate to the shoots above [Plate 2; Fig. B], by the mid second or third week. Full length of the plant, that is, with the well developed root and shoot system was observed by the forth week.

As hardening and acclimatization is the crucial necessity of *in vitro* regenerants, these plantlets were subjected to hardening process. They were then taken for soil survival experiments [Plate 2; Fig. C]. These plants were able to reach maturation and even flowered, giving a successful demonstration of tissue culture technique within time period of 35-45 days

The result thus proved very significant in successfully obtaining regeneration in just one step and their further survival in soil made the micropropagation successful.

In *T. ammi* regenerants were obtained via shoot tips inoculated on MS medium supplemented with auxin IBA [Plate 3; Fig. A]. Further increase in the shoot length as well as emergence of new shoots were obtained on the same medium. The shoots grew vigrously and by the second week development of root system was also attained. Thus, complete plantlets with well differentiated root and shoot system were ready for transfer to the pots within three weeks time period [Plate 3; Fig. B]. The regenerated plantlets after hardening were transferred to the field [Plate 3; Fig. C].

These experiments thus established a model system for quicker plant regeneration on auxin alone in a single step, eliminating the requisite of a separate rooting medium in the candidate crops.

Efforts were also directed towards multiple shoot production from the shoot tip explant in case of *T. ammi*. However, the shoot tip explants for these particular set of experiments were excised from seedlings raised on NAA-BAP containing MS media. These explants yielded better results as compared to those excised from seedlings raised on paper bridges or on phytohormone devoid synthetic media.

These shoot apices when inoculated on MS medium, containing BAP in conjugation with IAA, proliferated into a large number (12-16) of multiple shoots within one week of inoculation [Plate 4; Fig. A]. After three weeks of incubation the shoots were separated individually and cultured on IBA containing medium [Plate 4; Fig. B]. This nutritional constitution initiated rooting, at the same time inducing a few more new shoots, thus establishing a protocol for repetitive multiple shoot production.

Moreover, in order to improve the above protocol for multiple shoot prodcution suspension culture was also tried using the shoot tips from seedlings raised on NAA-BAP containing medium. Most significant results were obtained when the shoot apices were cultured in agitated liquid MS medium containing very high levels of auxin IAA in conjugation with very low concentrations of BAP (less than 1.0 mg/l) [Plate 4; Fig. C], where in, the shoot tips proliferated to give rise to multiple shoots (6-10) within a week. The shoots grew vigrously to their complete length within 10-15 days. The shoots for their further elongation and rooting were then separated individually and transferred to solidified MS medium incorporated with IAA or IBA singly in the full strength MS medium [Plate 4; Fig. E].

Thus, complete plantlets were obtained just within 25-35 days of culture and could survive in the field conditions [Plate 4; Fig. D; Plate 5; Fig. A-B].

Hence, tissue culture has been affirmed as a means to cultivate plant parts whether a single cell, tissue or an organ under aseptic conditions to overcome many problems of conventional agricultural practices. Moreover, tissue culture system also appears as an ideal system which helps in investigating physiological biochemical and genetical structural problems related to plants.

Thus, studies with plant cell cultures clearly have bearing upon a variety of problems still unsolved at the grass root level as well as for their applied research. In the application of *in vitro* methods for the improvement of the genetic potential of plants, for their oil content and medicinal values, the regeneration of plant attains special significance. Although totipotency is probably characteristic of all plant cells, but it's expression may be limited to particular cells. It is apparently confined to cells that are identified as meristemoids. Meristemoids are often located in specific tissues and organs. Murashige and Nakano, way back in 1967 also reported that tissues excised from the more recently produced parts of a herbaceous plant are more regenerative than those from older regions. Higher regenerative ability of meristematic cells has also been reported by Gamborg *et al.*, 1974 as well as Mukhopadhya and Bhojwani, 1978. This implies to the fact that perhaps the most important determinant of plant multiplication and quality of regenerated plants is the initial explant. In the absence of a suitable explant success is at best limited. Murashige, 1974 also mentioned plant regeneration accomplishment from various explants *viz.*, leaves, stem, cotyledons, microsporophytes, as well as shoot tips. Reports are also available which favour use of diverse explants such as hypocotyl (Meiners *et al.*, 1991; Schroeder and Stimart, 1997; Audichya, 1999), cotyledon (Knittel *et al.*, 1991; Chraibi *et al.*, 1992; Sharma, 1996), cotyledonary node, (Meiners *et al.*, 1991; Distabanjong and Geneve, 1996; Sharma, 1999), nodal segment (Lakshmi Sita, 1986; Hussein, 1997) in various plant species.

However, during our experimentation with the candidate crops, shoot tips emerged as the most amenable explant as also supported by Luckner *et al*., 1984; John and Batra, 1994; Sardana, 1998.

The next determinant for plant regeneration is providing optimum provisions of nutrient formulation.Studies have been conducted on the nutritional factors by Singha *et al*., 1987.

According to Skoog and Miller, 1959 the relationship of auxin – cytokinin balance to root and shoot initiation remains at the seat of plant regeneration. The suitability of shoot tip explant for regeneration and it's sensitivity to various hormones is due to the activity of meristematic cells, which are actively dividing and are known to have dense cytoplasm with much more uniform and homogenous composition. Present studies on two medicinal plants revealed that when auxin used singly proved most effective.

However, Murashige (1974) developed the concept of developmental stages for micropropagation mentioning 3 stages *viz*:

Stage 1: Explant establishment
Stage 2: Multiplication of the propagules
Stage 3: Rooting and hardening for planting into soil. This concept stimulates the awareness that a single medium usually is not sufficient for *in vitro* plant multiplication and regeneration. Transferring the propagules through a series of specially designed chemical and physical environment holds the key to success.

Though, sometimes deviation from this above concept are also observed, like in the present work, where complete plant regeneration with shoot elongation and rooting was obtained on the same medium composition in both the crops. Herrera *et al*. (1990) for the first time reported similar kind of a technique in *Digitalis thapsi*, eliminating separate medium requisite for rooting.

Auxins are usually required for shoot growth, but in the present case both *Cumin* and *Trachyspermum*, IAA proved effective in inducing rhizogenesis. However, rhizogenic effect of IAA was contradicted by the report of Thimann (1977) who attributed the inhibitory effect of auxin on root elongation. Nevertheless, several reports (Jha *et al*., 1983; Bajaj and Mahopatra, 1987; Gulati and Jaiwal 1990; Dave, 1994; Ajita, 1996) are available in support of the use of auxins, particularly IAA.

In case of ajowain multiple shoot production was also accomplished from the shoot tip explants cultivated on cytokinin (BAP) containing nutrient medium. Reports on *Brassica* (George and Rao, 1980); *Carthamus* (George and Rao, 1982); *Sesamum indicum* (George *et al*., 1989; Gogna, 1993); *Syzygium aromaticum* (Mathew and Hariharan, 1990) also stand in consonance to this result.

Enhanced multiple shoot production in *Trachyspermum* was obtained during the experiments by germinating the seedlings *i.e.* the source of explant on MS medium incorporated with an auxin and cytokinin (NAA-BAP). George and coworkers in 1987 also reported presoaking of *Sesamum indicum* seeds and their subsequent germination on cytokinin containing media. Jain and Datta (1992) also studied the effect of presoaking the explants on organogenesis.

Thus, present investigations reflect upon shoot multiplication facilitated by exogenous cytokinins with low concentration of auxin indicating presence of endogenous auxins in the shoot tip explants. This indicates that shoot tip being the principal site for auxin biosynthesis, which may produce multiple shoots where cultured on cytokinin alone.
Another remarkable aspect observed was the effect of suspension medium on multiple shoot production.

Miller and Murashige (1976) mentioned that choice between gelled and liquid formulations should not be made arbitarily as it may affect the plant growth, multiplication as well as survival. This view holds true in the present plant systems, where the complete system of shoot multiplication in *Trachyspermum* was accelerated by one week. Snir and Erez, 1980 also reported faster growth rate of apple shoot tips on liquid medium. The use of liquid medium for *in vitro* culture has been considered an ideal technique for mass production as it reduces manual labour and facilitates change of medium. Consequently this technique has been employed by Teisson and Alvard (1995) on a number of species *viz.* Coffee, *Heavea*, *Musa*.

In all the cases root induction was obtained on sole auxin containing media.

Role of auxin for rooting has also been supported by the reports of Lakshmanan and Dhanalakshmi, 1990; Gogna, 1993; Rout *et al.*, 1997; Bhuchar *et al.*, 1999. When well developed shoots are produced, they are separated and cultured for rooting.

However, the plantlets during their *in vitro* cultivation grow under special climatic and light conditions, making them very sensitive. Thus arises the need for their hardening and acclimatization for their adaptation to an environmental change. Keeping this in view, during the present investigation *in vitro* produced regenerants were maintained under high humidity conditions for initial two weeks with their growing in a mixture of soil and vermiculite. These are then gradually exposed to the natural climatic conditions. Similar hardening process has been used by Kaul, 1987; Batra, 1998; Sharma, 1999.

Thus, to conclude, plant cells remain an important source of medicinal compounds and account for over a quarter of all prescribed drugs with an annual market value of over $ 3 billion (Venkataraman, 1998). In view of this, as well as the growing awareness about the side effects of the synthetic drugs, has provided the necessary impetus to hasten the pace of research in medicinal plant biotechnology.

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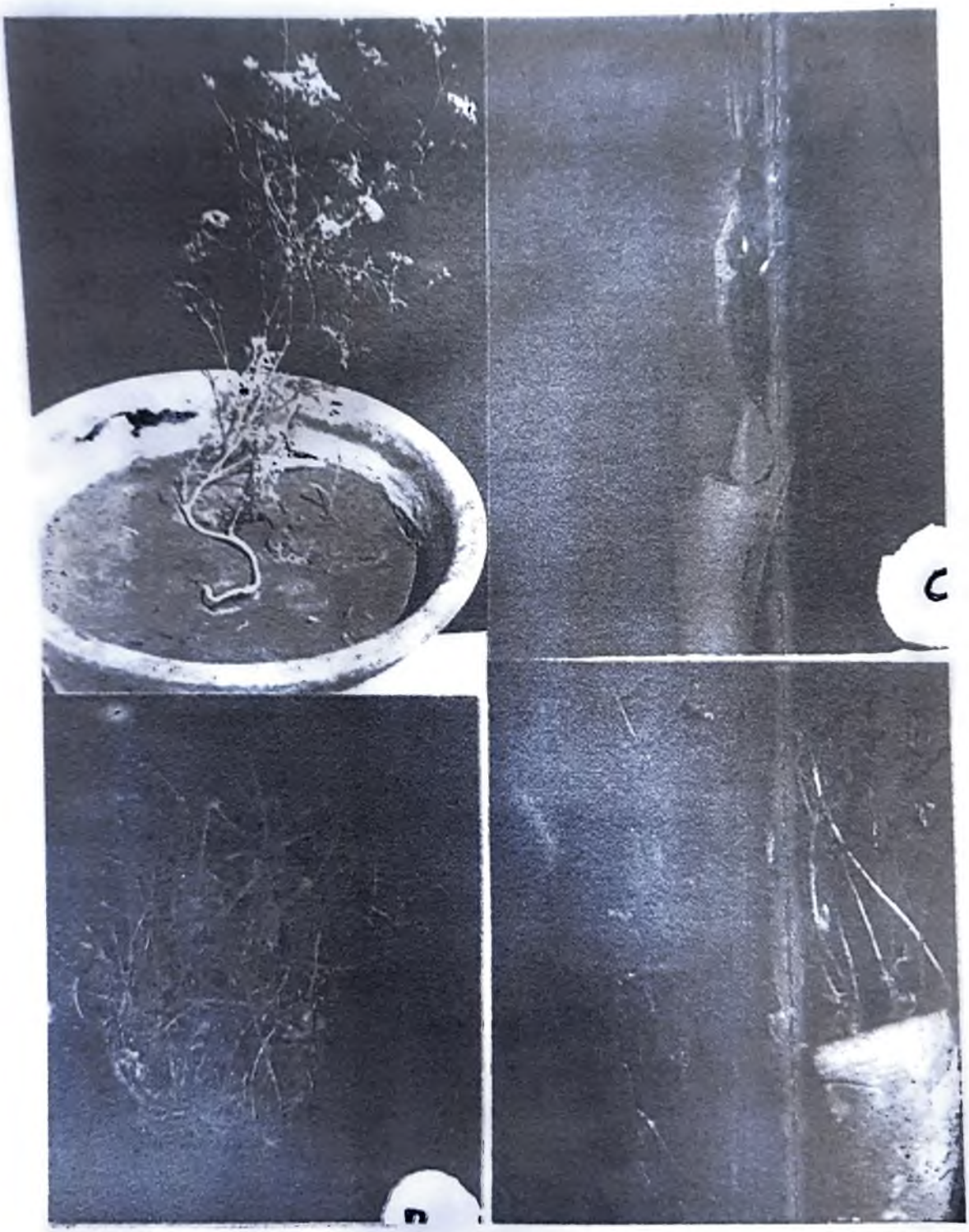


Aseptically grown seedlings of *Trachyspermum ammi* and *Cuminum cyminum*

Fig. A Germinated seeds of Cuminum cyminum

Fig. B Germinated seeds of *Trachyspermum ammi*

Fig. C *In vitro* germinated seeds of Cuminum cyminum on paper bridge

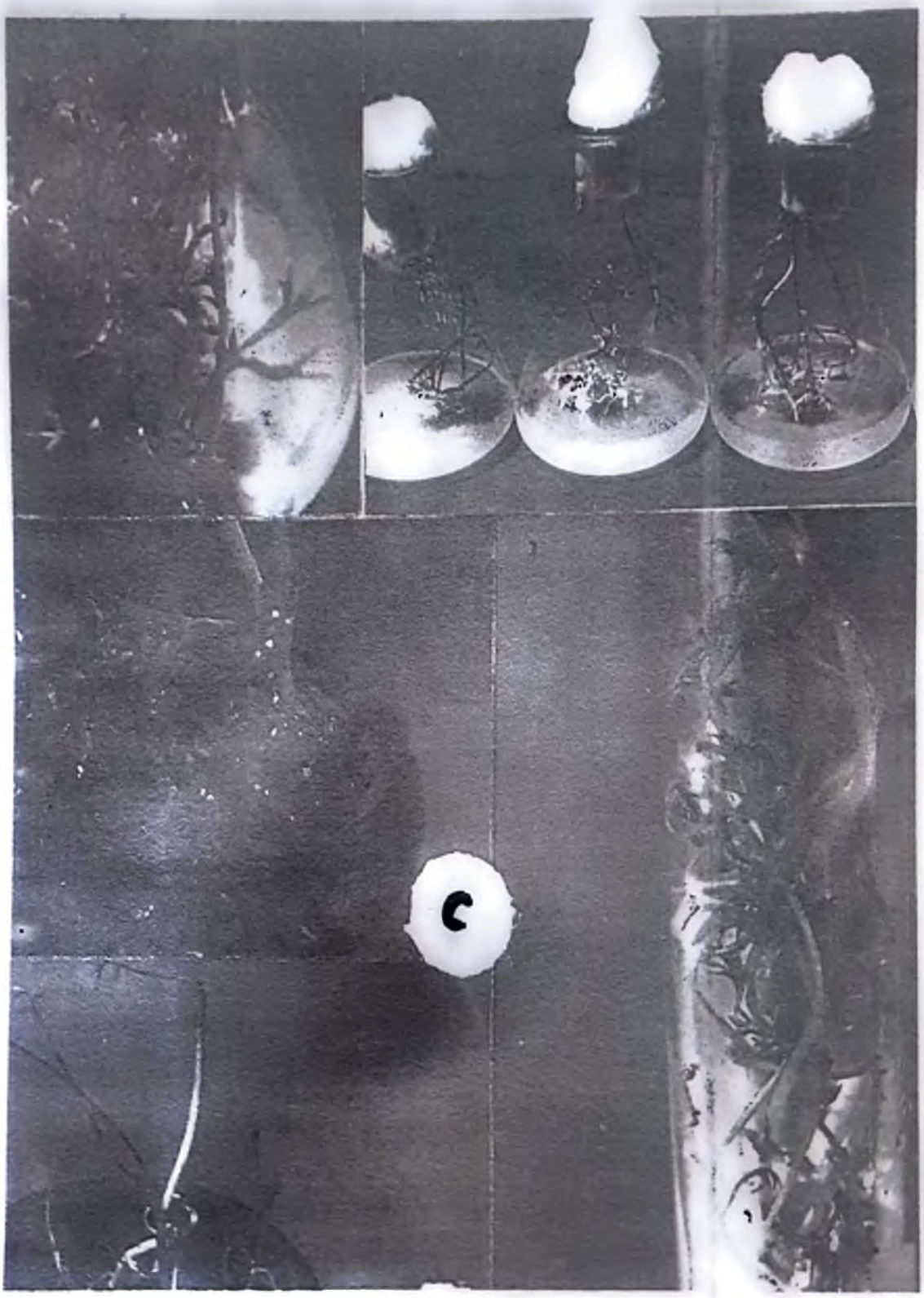
Fig. D *In vitro* germinated seeds of *Trachyspermum ammi* on paper bridge

Shoot tip culture leading to complete plant regeneration in *Trachyspermum ammi*.

Fig. A — *T. ammi* regenerants on MS medium + BA

Fig. B — Root initiation

Fig. C — Pot transfer



Rapid shoot multiplication in T. ammi.

Fig. A Multiple shoot proliferation

Fig. B Transfer of separated shoots on IBA

Fig. C Multiple shoot proliferation in liquid medium containing BAP and IAA

Fig. D Separated shoots transferred to solid media for rooting

Fig. E Plantlet transferred to soil

Fig. A Acclimatization of *in vitro* regenerated plantlets of *L. cumini*
Fig. B Pot transfer

Shoot tip culture leading to complete plant regeneration in Cuminum cyminum in one step.

Fig. A Growth of explant after inoculation
Fig. B Root induction
Fig. C Complete plantlets transferred to soil

ROLE OF BIOTECHNOLOGY
in
MEDICINAL AND AROMATIC PLANTS
Volume-X

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HYDERABAD

CANCER : HORNEST NEST OF MEDICAL SCIENCES

AMLA BATRA, SHILPA RAJORE,
MANISHA SHARMA AND DINESH JALOOTHARIA

Chapter outline

1. INTRODUCTION

Today it is almost impossible for anyone to escape a personal experience with cancer for it strikes in two out of every three families. It is a ravaging disease which consumes one's flesh and invades internal organs to produce death. Cancer is a group of disease characterized by the disregulate proliferation of abnormal cells that invade and disrupt surrounding tissues. Being a Major cause of death its social and economical impact is overwhelming (Chatterjee *et al.*, 2002).

Cancers, differ from normal tissues. They grow more-or-less autonomously, beyond host ability to control them. They spread into and destroy surrounding tissues. They detach fragments of themselves, which travel throughout the host's body, and in new locations, lodge and begin new cancers. They derange the host's metabolism and cause "wasting". Finally, if untreated, they kill their host and when they do, die as well. No normal tissue exhibits such bizarre behaviour.

2. HISTORY

Cancer is by no means a new disease. The people of Egypt and India, over four thousand years ago, were afflicted with the same malignant growths. Around 400 B.C., "Hippocrates, the father of Medicine", called these rapidly growing swellings *Karkinomas*. It is from this origin that the modern term *Carcinoma,* which refers to all cancers of the epithelial or living

tissue of the body, is derived. But is was Galen, personal physician to the Emperor Marcus Aurelius, who coined the term cancer, which literally means "a crab", over 1800 years ago he observed that, "Just as a crab's feet extend from every part of its body, so in this disease the veins later discovered to be lymphatic vessels are distended and form a similar figure".

Paul of Aegina (A.D. 625-690) four centuries later repeats this comparison, but modifies it by adding the following.

"However, some say that cancer is so called because it adheres with such obstinacy to the part it seizes that, like the crab, it cannot be separated from it without great difficulty".

In support of Paul's view Haddow (1936) mentioned the application of the term "crab" to various grasping tools whose invention was prompted by the crab's powerful chelae, but also recalled an intriguing alternative explanation, advanced by Louis Westenra Sambon, in the frequent parasitic association between crabs and the tumour-like *Sacculina carcini*. This parasite in the Cypris stage attaches itself to the body of a young crab and, after shedding "every part of its economy save a small bundle of all-important cells", enters the host and becomes the *Sacculina interna*, which proceeds to absorb nourishment by means of branching suckers extending like roots to every portion of the crustacean's anatomy.

Echoes are still heard of the fantastic superstition that there is a connexion between Cancer, the sign of Zodiac, and cancer, the disease for some people still believe that those born under that sign are predestined to die of cancer.

3. ORIGIN OF CANCERS

Cancer is neither new nor uniquely human. Malignant growth may well be as old as life itself. Tumors have been described in nearly all forms of life in the animal kingdom and neoplastic growth is well known in plants. Among humans, cancer has been noted in mummies preserved from ancient Egypt, and no reason exists to suppose it began there. Its distribution includes nearly all life forms on this planet, and it is certainly not new.

Cancer is a disease associated with aging. In former days, many diseases claimed people's lives, frequently before they could become old. People now live longer and consequently may fall victim to cancer. The incidence of certain cancers has changed, some increasing, others decreasing. Additional use of carcinogenic substances may account for the increased incidence of certain cancers, in particular, those arising in the lung. But others, like those of stomach or uterus, for unknown reasons, have decreased substantially.

Cancer is a disease that has been socially unacceptable. Only recently have victims begun to disclose their illness, and even now a residue of the older attitude persists. Consider, for example, the awe engendered when Mrs. Gerald Ford, wife of the then President of the United States, announced publicly that she had breast cancer. Many congratualted her on her *courage* at having made such a public statement and expressed the hope that her example would encourage others to overcome their inhibitions and would seek help early. With more

people talking freely about cancer, there may be a perceived rather than real increase in incidence.

In a sense, however, it makes little difference whether cancer incidence is increasing in fact or in appearance. Cancer is a dreaded disease which the public wants to cure. It is high on the list of national priorities and, for that reason alone, is a medical problem of the first magnitude.

4. DISTRIBUTION OF CANCER

Neoplasms of many different sites and tissues occur in all species of animals that have been studied in sufficiently large numbers for a long period. They occur in lower forms such as amphibia and fish (Schlumberger *et al,* 1948), and at the same time many plants also develop a cellular reaction that appears to be analogous to cancer. This wide occurrence of neoplasms in nature excludes specific constituents of diets and other environmental exposures that man has developed in the process known as civilization from general implication as the only or the main factor responsible for cancer.

The term "spontaneous tumor" is used to designate neoplasms that appear without a known stimulus or agent being applied to the animal. In other words, they are tumors of unknown etiology.

Neoplastic diseases are found in all human populations that have been adequately studied. There are some striking racial and regional differences, however, in the occurrence of different types and sites of cancer.

5. BIOLOGICAL NATURE OF CANCERS

Scientists have discovered that cancers are composed of millions of abnormal cells which possess a malignant or life-threatening growth pattern. Since growth is a distinguishing biological characteristic of all living matter, to understand the nature of cancer one must first understand the functions of cells, the basic biological units of all plant and animal life in the normal growth process.

Individually, cells are so small that they are invisible to the naked eye. In fact, it would take 700-800 of these minute structures just to cover the head of a pin. Life begins when two of these, the female egg or ovum and the male sperm, unite. Almost immediately the fertilized egg begins to divide and forms new cells. As this cellular multiplication continues, tissues and organs are formed and growth continues until an adult human body composed of billions of cells is constructed. From the time of conception until the individual is fully developed, cellular division normally proceeds at a remarkable speed and is an orderly, controlled, and predictable fashion. At maturity, cellular production slows down and continues only at a rate sufficient to repair or replace the worn out and damaged cells. Cancer which may occur at any stage of development from infancy through adult life begins, when one or more of the billions of cells involved in this complicated and little understood growth process develop an immunity to the

biological forces which normally regulate growth. Endowed with extraordinary energy, these abnormal cells divide and reproduce at an extremely rapid rate but with no apparent end point. Eventually invasion of the surrounding tissues occurs and a progressively enlarging mass of cancerous tissue for which there is no room in the body is formed. When discovered, these life threatening, parasitic growths are referred to as malignant neoplasms or cancers.

The words "tumor and neoplasm" are used interchangeable in referring to any new growth of tissue which serves no function in the body. But not all neoplasms (or tumors) are malignant. In fact, the great majority are confined to one location, such as the breast or skin and never invade the surrounding tissues or spread to distant sites. These non lethal tumors are said to be benign.

6. TYPES OF CANCER

A common misconception is that cancer is one disease. Actually, there are over a hundred different types of cancer, which are classified according to their site of origin and their microscopic appearance. These may and do originate in all parts of the body and from practically all of the different cell types which form the various internal organs. In order to simplify matters, however, all cancers are separated into the following four subgroups, each of which indicates the type of body tissue from which the cancer originated:

- *Carcinoma,* a malignant tumor of epithelial or lining tissue (skin, various membranes, and glandular tissue).
- *Sarcoma,* a malignant tumor of connective tissue (bone, muscle, and other "supportive" tissues).
- *Lymphoma*, a malignant tumor of lymphatic tissue (Hodgkin's disease and lymphosarcoma).
- *Leukemia,* a malignant disease of the blood-forming tissues (often referred to as "cancer of the blood").

It is also important that the specific types of cancer within each of the above subgroups possess their own unique growth pattern and degree of virulence. Consequently, not only must each cancer be treated differently, but the response to therapy may be extremely variable.

7. CAUSES OF CANCER

Many factors are believed to increase a person's chances of developing Cancer. Skin cancer frequently occurs after over exposure to sunlight, to x-rays, or to radium. Smoking is now undeniably associated with the development of lung cancer. Exhaustive studies involving the Japanese survivors of the atomic blasts of World War II have demonstrated that excessive exposure to atomic radiation is unquestionably linked to the development of leukemia, a fatal cancer of the blood. Employees in aniline dye factories are known to haven increased tendency to develop cancer of the urinary bladder.

8. CHEMICAL AND PHYSICAL CARCINOGENS

The agents that are capable of eliciting a neoplasm usually are designated as carcinogenic (Table 1).

Table 1 : Physical and chemical agents associated with cancer formation

Agent	Sites of cancer
Tabacco	Lung, oral cavity, tongue, larynx, bladder
Alcohol (heavy consumption)	Oral cavity, tongue, and larynx
X-ray and radium	Lung, skin, blood
Radioactive chemicals	Bones, nasal sinuses, thyroid
Sunlight	Skin
Inhalant exposure to :	Lung
Asbestos	
Nickel	
Chromates	
Radioactive ore and gas	
Prolonged contact with :	Skin
Petroleum products	
Arsenic	
Tar	
Soot carbon black	
Aromatic amines, e.g.	Bladder
Aniline days	

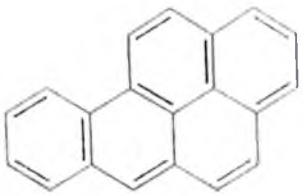
Several carcinogenic agents were known from clinical experience long before the extension of the investigations to the laboratory. Perhaps the first was the description by Pott, 1775, of scrotal carcinoma in men exposed to constant contact with soot. In 1915, (Yamagiwa and Ichikawa, 1918) reported that continuous painting of rabbit's ears with tar led to the appearance of carcinoma. The observation was rapidly extended to the mouse, and the simplicity of the method led to its extensive use in cancer research.

Polycyclic hydrocarbons

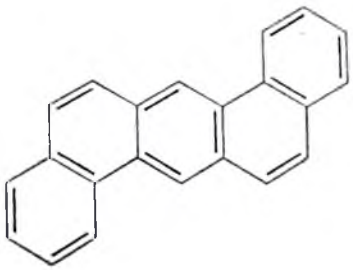
The successful search for the active constituent in tar was the achievement of the British group under the leadership of Kennaway and Cook. The active ingredient was found to be benzpyrene. As a matter of fact, the first carcinogenic polycyclic hydrocarbon compound to be described, in 1930, was dibenzanthracene (Kennaway *et al.*, 1955). Further modifications of the benzanthracene nucleus led to the synthesis and biologic testing of numerous related compounds. Particular interest aroused when one of the more active of the carcinogenic hydrocarbons, methylcholanthrene was synthesized from bile acids. The structural molecular resemblances between carcinogenic hydrocarbons, cholesterol, bile acids and steroid hormones that also were being isolated and synthesized during this period stimulated hopes that a

common molecular structure and the physiological elaboration of the body of compounds similar to the hydrocarbons could clarify the cancer problem. Carcinogenic hydrocarbons act at point of contact.

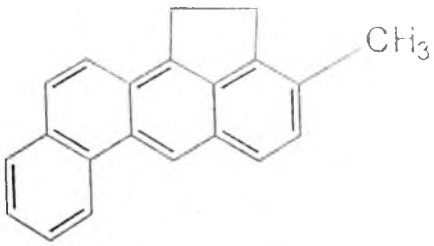
In man, exposure is usually to crude mixtures of materials, so that incrimination of single chemicals is difficult. Nevertheless, compounds of the polycyclic hydrocarbon type probably are the active carcinogens in the industrial skin cancers of workers with coal tar, pitch, soot, asphalt, shale, petroleum and paraffin oils (Hueper, 1942). Similar compounds also are important in the production of cancer of the lung, larynx, and oral cavity among tobacco smokers (Surgeon, 1964) and in the increased incidence of respiratory cancers among city dwellers exposed to atmospheric pollutants.



Benzo[a]pyrene
(3,4-Benzpyrene)



Dibenz[a,h]anthracene
(1,2,5,6-Dibenzanthracene)

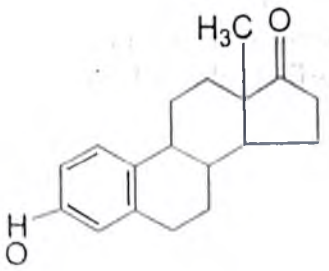


3-Methylcholanthrene

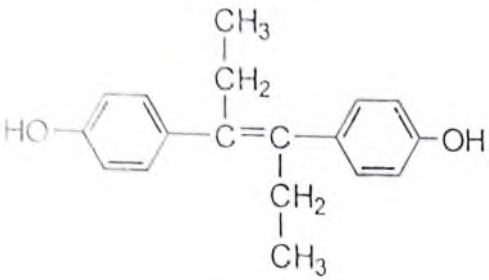
Estrogens and other hormones

Estrogens are among the chemical compounds whose carcinogenic action is distant to the site of administration and limited to specific target tissues. Estrogens include synthetic chemicals such as diethylstillbestrol and triphenylethylene as well as physiologically produced chemicals with estrogenic activity.

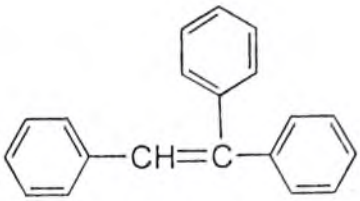
The carcinogenic effects of estrogens in rodents was demonstrated much later for man. In 1971, (Herbst *et al.*, 1971) reported the occurrence of cancer in the vagina in girls whose mothers had taken diethylstilbestrol in large doses during their pregnancy. Thus, this synthetic, orally effective estrogen is carcinogenic for the human fetus, with the effect becoming evident fifteen years later. In 1975, it was shown that exogenous estrogens increase the risk to endometrial carcinoma.



Estrone



Diethylstilbestrol



Triphenylethylene

Nitrosamines and related compounds

The nitroso compounds, such as dimethylnitrosamine include active and multifarious carcinogens. They are potential industrial and environmental hazards to man. Minute amounts can be formed in the stomach from nitrites and amines in the diet (Greenblatt *et al.*, 1971), raising the possibility of their role in the occurrence of gastrointestinal cancer in man.

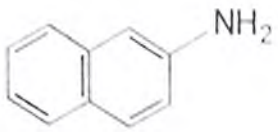
Other chemicals and materials

Ethyl carbamate (urethane) produces pulmonary tumors and hepatomas. It is also an "incomplete" carcinogen for the skin in that it will evoke skin carcinoma if the site is also painted with croton oil an irritant with little or no carcinogenic activity (Roe *et al.*, 1995). The alkylating agents used in cancer chemotherapy are also carcinogenic (Shimkin *et al.*, 1966).

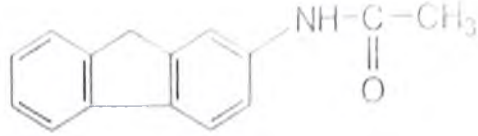
A number of inorganic chemicals are incriminated in the production of cancer in man. These include arsenic, which produces cancer of the skin following extended medicinal or industrial exposures; chromates, which, upon inhalation produce bronchogenic carcinoma; and nickel, which increases the occurrence of bronchogenic carcinoma and carcinoma of the nasal cavity (Demerece, 1948). Sarcomas have now been elicited in rodents with chromate and nickel compounds, but arsenic remains to be demonstrated convincingly as carcinogenic in animals (Hartwell, 1951). Asbestos is incriminated as a carcinogen in man as well as in animals (Selikoff *et al.*, 1968).

Carcinogens also are recovered from natural sources, such as plant foodstuffs, and contaminants thereof. Discoveries of aflatoxin and of cycasin are pivotal.

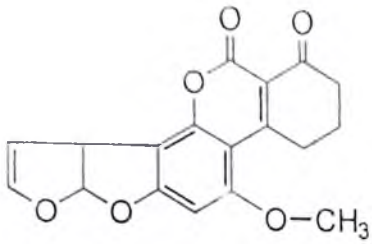
Cycasin is a natural product of the *Cycas circinalis* nut, a nutritional source in Guam and other tropical regions. The active chemical is a glycone and is not carcinogenic unless the glycone portion of the molecule is first split off by intestinal flora, yielding the absorbable aglycone methylazoxymethanol.



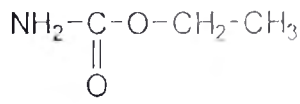
2-Naphthylamine



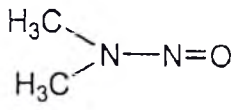
N-2-Fluorenylacetamide
(2-Acetylaminofluorene)



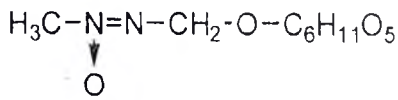
Aflatoxin B_1



Ethyl carbamate
(Urethane)



Dimethylnitrosamine



Methylazoxymethanol-
β-glucoside cycasin

Roentgen and ultraviolet radiations

The fact that roentgen rays and radium are carcinogenic was shown within ten years of their discovery by the tragic occurrence of skin carcinomas in physicians and other workers who exposed themselves to the new rays. These ionizing radiations are carcinogenic, whether delivered from external sources or administered in the form of fission products.

The tragedy of Hiroshima established that ionizing radiations are also leukemogenic and induce thyroid cancer in man. Therapeutic and even diagnostic doses of radiations increase the risk to leukemia, indicating that ionizing radiations may become an increasingly important source of carcinogenic exposure for the populations of the future (Glucksmann *et al.*, 1957).

The induction of skin cancer following exposure to ultraviolet radiations was first suspected on the basis of clinical experience and subsequently reproduced experimentally in mice. The effective wavelength was found to be in the 2,900 to 3,200 Å range. The production of tumors depends upon the quantity of radiant energy applied rather than upon its intensity and

a quantitative relationship, has been established between the dose of radiations and the neoplastic reaction (Blum, 1959). A different type of action may be involved in the induction of neoplasia by ultraviolet radiations and carcinogenic hydrocarbons, since the action of these two agents is not additive.

9. PLANT ALKALOIDS

Vinca alkaloids

Vincristine and vinblastine are complex alkaloids derived from the periwinkle plant *Catharanthus roseus* (also called *Vinca rosea*). They are members of a general class of drugs that act as mitotic inhibitors ("spindle poisons"). Although several vinca alkaloids have been isolated and shown to be cytotoxic, vincristine, vinblastine, and vindesine are the only ones used clinically. The mechanism of action of these drugs has been reviewed by Wilson *et al.*, 1970.

The mitotic inhibitors act by interfering with the function of microtubules. The cytotoxicity of vincristine and vinblastine is attributed to their ability to interrupt cell division in metaphase (Bruchovsky *et al.*, 1965), but other effects could also contribute to cell death. Their action is M phase specific. If the drug is removed shortly after metaphase arrest, the effect is reversible and many cells will proceed through the growth cycle (Malawista *et al.*, 1968). Indeed, this type of blockade and reversal can be used to obtain synchronous cell populations.

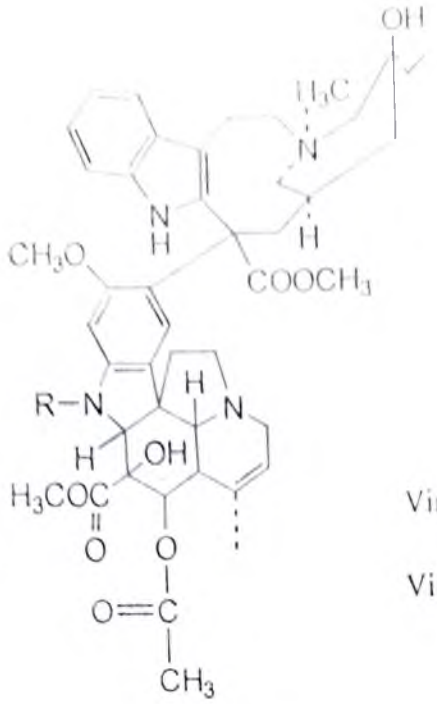
Use

Vincristine is one of the drugs used to treat patients with advanced Hodgkin's lymphoma as part of the preferred MOPP regimen [mechlorethamine, vincristine (Oncovin), procarbazine, prednisone] (De Vita *et al.*, 1972). Vincristine is used in various combination regimes to treat acute myelogenous leukemia, lymphocytic lymphoma and diffuse histiocytic lymphoma. Vincristine is sometimes used to treat adult solid tumors, such as those of the breast, lung, and cervix.

Vinblastine is also used in combination drug therapy to treat several lymphomas, including advanced Hodgkin's disease. Vinblastine is sometimes used alone in therapy of patients with gestational choriocarcinoma that is resistant to methotrexate and in combination with other drugs to treat patients with breast cancer that is unresponsive to hormonal therapy and resistant to the major preferred combination drug regimens.

Toxicity

Vinblastine depresses the bone marrow. Vincristine depresses the bone marrow much less commonly and it is considered to be marrow sparing, compared to most anticancer drugs. It is possible that this difference could be due to more efficient uptake of vinbalstine by the stem cells of the marrow, although this has not been demonstrated. Vinblastine can produce thrombocytopenia and anemia, but these occur rarely, and in clinical use, vinblastine is considered to be platelet sparing. Vincristine may actually produce thrombocytosis in some patients (Carbone *et al.*, 1963).



	R
Vincristine	CHO
Vincristine	CH_3

Taxol

The naturally occurring complex diterpenoid taxol (Taxol A, taxol, paclitaxel) (1) (Wani *et al.*, 1971) has recently been identified as an exceptionally potent novel chemotherapeutic drug to combat cancer. The compound has been considered by the National Cancer Institute (NCI) as "the best anticancer agent developed in recent years". The discovery of the compound in 1966 ranks in retroprospect as one of the most significant discoveries ever made in the field of naturally occurring anticancer drugs (Kingston, 1991).

Taxol (1) (Wani *et al.*, 1971) is a novel complex molecule with various functionalities in different chiral centres. It is available from natural source (yew species) in very low yield and its synthesis is very difficult (Das and Das, 2000). The compound is highly potent for treatment of ovarian and breast cancers (Rowinsky *et al.*, 1990). It exhibits a unique mechanism of action as a microtuble stabilizing agent. Such a mechanism was previously not shown by any other known anticancer agents (Schiff *et al.*, 1979). Taxol has recently attracted the attention of the chemists and biologists all over the world mainly due to the following reasons :

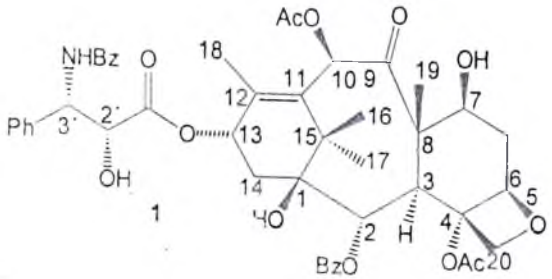
- natural scarcity
- chemical complexity
- promising antitumour activity
- unique mechanism of action.

The molecule contains an unusual oxetane ring and a phenylisoserine moiety as a side chain (Wani *et al.*, 1971). The yew plants (*Taxus*) of different varieties and of different regions

were subsequently investigated to determine their taxol content. The compound has been found in all the *Taxus* species and in the endophytic fungi of *Taxus brevifolia* (*Taxomyces andreana*) and *Taxus wallichiana* (*Pestalotiopsis microspora*) (Stierle *et al.*, 1993; Strobel *et al.*, 1996). However, the bark of the Pacific yew is the best natural source of taxol. It has also been observed that taxol concentration in the plant is highest in the bark, with roots second best, followed by needles and wood (Das and Das, 2000).

Different analogues of the compound in which the N-benzoyl group of the side chain, replaced with other acyl groups have been synthesized and one such analogue, taxotere (docetaxel) (2) (Guenard *et al.*, 1993; Schrijvers and Oosterom, 1996) has been found to be more impressive than taxol. Acid catalyzed conversions and decomposition of taxol have also been thoroughly studied (Das *et al.*, 1998; Das *et al.*, 2000).

The semisynthesis of taxol has been carried out (Denis *et al.*, 1988) from 10-deacetylbaccatin III (3), a major taxoid constituent of *Taxus baccata* and other yew plants. The total synthesis of taxol has also been achieved by various researchers.



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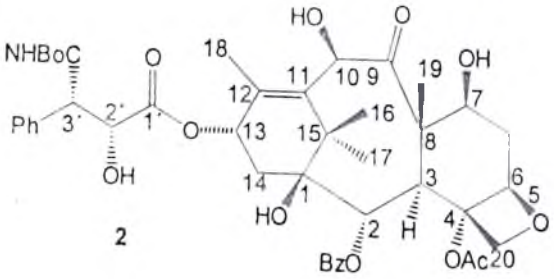

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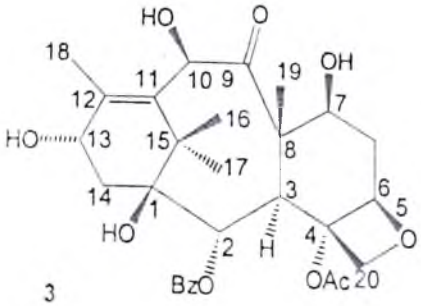


3

Bioactivity

Taxol (1) has emerged as a highly promising cancer chemotherapeutic agent (Das and Das, 1994). The compound at first showed potent cytotoxicity against KB cells and subsequently

its antitumour activity was observed in different leukemia models such as L 1210, P 1534 and P 388. The activity was confirmed *in vivo* in Walker 256 carcinosarcoma and B 16 melenoma systems. The compound was also found to be highly active in some new bioassays including human-tumour-xenograft assays (Kingston, 1994).

Taxol has been established as a novel antimitotic agent with a unique mechanism of action on the tubdinmicrotubule systems. The compound was found to stabilize microtubules and inhibit depolymerization back to tubulin. This is the opposite effect of other known antitumour agents which all bind to soluble tubulin to form microtubules (Schiff *et al*., 1993; Parness and Horwitz, 1981).

Taxol showed excellent activity against several human cancer diseases such as ovarian, melanoma and breast cancer (McGuire *et al*., 1989; Holmes *et al*., 1991). The compound was approved by the US Food and Drug Administration (FDA) for the treatment of refractory advanced ovarian cancer and metastatic breast cancer in 1992 and 1994, respectively (Kingston, 1994).

Epipodophyllotoxin analogs

Podophyllotoxin is synthesized by the plant *Podophyllum peltatum*, commonly known as the American mandrake or May apple. It is a mitotic inhibitor that acts by binding to tubulin. A number of semisynthetic derivatives of podophyllotoxin are now available and two of them, VM 26 and VP 16-213, are active against some animal and human cancers; they are now in clinical trial in the United States. The antitumor activity, pharmacology, and toxicity of these epipodophyllotoxin analogs have been reviewed (Rozencweig *et al.*, 1977).

VM 26 and VP 16-213 do not cause dissolution of microtubules (Krishan *et al.*, 1975) and they reduce the mitotic index, rather than produce mitotic arrest. These drugs appear to have their primary effect in G_2 or perhaps in late S phase and they prevent the entry of cells into mitosis (Krishan *et al.*, 1975; Grieder *et al.*, 1974). In various systems, the drugs have been shown to inhibit mitochondrial electron transport (Gasalvez *et al.*, 1972) to decrease nucleotide uptake into cells (Loike and Horwitz, 1976) and to increase intracellular DNA degradation (Loike and Horwitz, 1976) but their biochemical mechanism of action has yet to be elucidated.

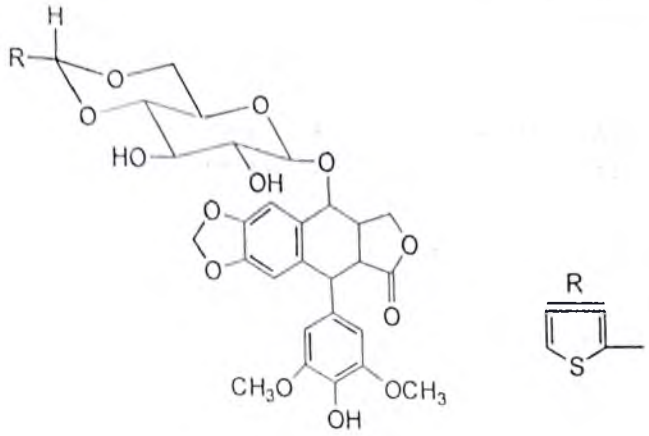
Use

The epipodophyllotoxin analogs are active against Hodgkin's disease, non-Hodgkin's lymphomas, acute leukemias, small cell lung cancer and central nervous system malignancies (Rozencweig *et al.*, 1977). Their relative lipophilicity and their activity against intracerebrally inoculated L1210 leukemia in mice have made these compounds attractive candidates for clinical trial in cancer of the central nervous system.

Toxicity

The dose-limiting toxicity for both drugs is leukopenia, with thrombocytopenia being somewhat less frequent (Rozencweig *et al.*, 1977). Chemical phlebitis can occur at the injection site. Nausea, vomiting and a reversible alopecia are common, but diarrhea is infrequent. Stomatitis, fever, chills, and episodes of generalized erythema, bronchospasm, and anaphylaxis have

been reported with these drugs. These drugs should probably not be given to humans by the intraperitoneal or the intrapleural route. No significant difference in actions, clinical effect, or toxicity has been demonstrated between VM 26 and VP 16-213 (Rozencweig *et al.*, 1977).



Epipodophyllotoxin analogs
VM 26 (NSC-122819)
VP 16-213 (NSC-141540) CH_3

10. ENZYMES

L-Asparaginase

Enzymes are used both locally and systemically in medicine, and several have been tested in experimental systems for possible anticancer activity. L-Asparaginase is the only enzyme now used clinically in the treatment of cancer.

Tumor cells that are killed by L-asparaginase have either no asparagine synthetase activity or very low levels of synthetase (Broome and Schwartz, 1967) which catalyze the transfer of an amino group to aspartic acid to form asparagine.

Asparagine synthetase activity has been assayed in asparaginase-resistant lymphoma sublines and found to be much higher than that of the asparaginase-sensitive parent cells (Broome and Schwartz, 1967). The mechanism by which asparagine depletion causes the lysis of sensitive lymphocytes has not been worked out. As might be expected, asparagine depletion is rapidly followed by inhibition of protein synthesis (Ellem *et al.*, 1970). Nucleic acid synthesis is inhibited later, presumably as a consequence of protein synthesis inhibition.

Use

Asparaginase has a very limited spectrum of clinically useful action. In 10 to 20 percent of patients with acute leukemia of nonlymphocytic nature, complete or partial remission has been reported with aspraginase therapy (Oettgen, 1975). No significant beneficial response has been reported with solid tumors and currently, asparaginase is essentially used only in

acute lymphocytic leukemia to induce remission. Because of the risk of anaphylactic reaction, it is generally not employed in maintenance therapy. Asparaginase has a minimal effect on the bone marrow and does not produce stomatitis and for these reasons it would be an ideal addition to combination drug protocols if it had a wider range of clinical activity.

Toxicity

Asparaginase therapy is commonly accompanied by nausea, vomiting, anorexia and fever (Haskell *et al.*, 1969). Early preparations of enzyme were contaminated with bacterial endotoxin but since purified preparations have become available, fever is somewhat less common. Because asparaginase is a foreign protein, hypersensitivity reactions would be expected; they have been observed in about 25 percent of patients (Zubrod, 1970). Many of the reactions are of the urticarial type but some patients experience an anaphylactic response. For this reason, a syringe with epinepherine should always be kept at hand during administration and patients should be carefully monitored.

11. MISCELLANEOUS ANTICANCER DRUGS

Hydroxyurea

The early studies of the biological activity and the pharmacology of hydroxyurea have been reviewed. The drug specifically inhibits DNA synthesis without inhibiting the incorporation of precursors into RNA or protein (Young and Hodas, 1964). When bacteria or mammalian cells are exposed to hydroxyurea, there is a marked reduction in the size of intracellular deoxyribonucleotide pools but no reduction in the amount of ribonucleotides (Skoog and Nordenskjod, 1971).

Hydroxyurea kills cells that are synthesizing DNA (Sinclair, 1967) and thus, like cytarabine, it is an S phase specific agent. It has been thought that the drug reversibly blocks the cell cycle at the G_1 side of the boundary between G_1 and S (Tobey and Crissman, 1972) but additional data suggest that G_1 cells treated with hydroxyurea enter the DNA synthetic period at a normal rate but that the rate of DNA synthesis is greatly reduced and the block is in the S phase (Walters *et al.*, 1976). Hydroxyurea is well absorbed from the gastrointestinal tract and it is routinely given orally.

Table 2 : Major untoward effects of the plant alkaloids and miscellaneous anticancer drugs and indications for their use.

	Principal toxicities		
Drug	**Acute**	**Delayed**	**Major therapeutic indications**
Plant alkaloids Vincristine	Localreaction after exravasation (avoid contact with skin and eyes)	Peripheral neuropathy (dose limiting); alopecia; bone marrow depression (marrow sparing relative to most anticancer drugs);	Acute lymphocytic leukemia (induction of remission); Hodgkin's lymphoma (e.g. MOPP regimen); acute

		hyperuricemia; constipation (adynamic ileus can occur)	myelogenous leukemia; non-Hodgkin's lymphomas; pediatric solid tumors; some adult solid tumor (e.g. brast, lung, cervix)
Vinblastine	Local reaction after extravasation; nausea and vomiting	Bone marrow depression is dose limiting (primarily leukopenia); alopecia; stomatitis; peripheral neuropathy (less common than with vincristine)	Hodgkin's and non-Hodgkin's lymphomas; testicular carcinoma; methotrexate-resistant gestational choriocarcinoma
Epipodophyllot-oxin analogs (VM 26, VP 16-213)	Nausea and vomiting; hypotension if administered too rapidly	Bone marrow depression; alopecia	Hodgkin's and non-Hodgkin's lymphomas; acute leukemias; small cell lung cancer; CNS malignancies
Miscellaneous drugs Asparaginase	Nausea and vomiting, fever; anaphylaxis	Hepatotoxicity, hyperglycemia; pancreatitis; abdominal pain; coagulation defect; CNS depression	Acute lymphocytic leukemia (induction of remission)
Hydroxyurea	Mild nausea and vomiting	Bone marrow depression; stomatitis; dermatological reactions	Chronic granulocytic leukemia; prevention of leukostasis in leukemia patients; malignant melanoma
Mitotane	Nausea and vomiting; diarrhea	CNS toxicity, including lethergy, dizziness, and visual disturbances; adrenal suppression; rash	Inoperable adrenocortical carcinoma
Procarbazine	Nausea and vomiting	Bone marrow depression; CNS depression; stomatitis; allergic reactions; disulfiram-like reaction with alcohol ingestion; monoamine oxidase inhibition (avoid sympathomimetic drugs and foods with high tyramine content)	Hodgkin's lymphoma (e.g., MOPP and CVPP regimens); non-Hodgkin's lymphomas; small cell lung cancer; malignant melanoma; brain tumors
Cis-platinum (DDP)	Nausea and vomiting	Naphrotoxicity; ototoxicity; bone marrow depression	Testicular tumors; ovarian and bladder cancers; head and neck carcinomas

Hexamethylmelamine	Nausea and vomiting	Bone marrow depression; peripheral neuritis; CNS depression	Ovarian and cervical cancer; lung cancer; lymphomas
Razoxane (ICRF 159)	Nausea and vomiting	Bone marrow depression; alopecia	Leukemias; lymphomas; colorectal carcinoma

Toxicity

Mild nausea and vomiting are experienced by most patients receiving this drug (Schwartz and Canellos, 1975). The major dose-limiting toxicity is bone marrow depression, with leukopenia and less commonly, thrombocytopenia and anemia. (Table-2). Megaloblastosis in the marrow is common. Stomatitis and gastrointestinal ulceration may occur when particularly large amounts of drug are given. Dermatological reactions in patients on long-term maintenance therapy include increased pigmentation, scaling and atrophy of the skin, partial alopecia, nail changes, and erythema of the face and hands (Kennedy *et al.*, 1975). Hydroxyurea is known to be teratogenic in animals, including primates and this effect must be considered when women of child-bearing age are treated (Wilson *et al.*, 1975). When hydroxyurea therapy is combined with radiotherapy, mucosal reactions in the radiation field may be more severe (Hussey and Abrahams, 1975).

Hydroxyurea

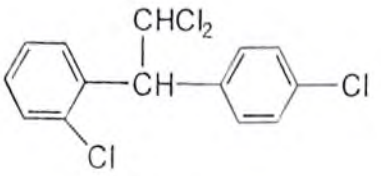
Mitotane

Mitotane is 1,1 dichloro-2 (*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane, best known by its trivial name, *o, p'*-DDD. Mitotane is used only in the palliative treatment of inoperable adrenocortical carcinoma (Lubitz *et al.,* 1973). The medical use of this compound is based on the observation that the insecticide DDD (an analog of DDT) produced necrosis and atrophy of the adrenal cortex in dogs (Nelson and Woodard, 1949). The isomer *o,p'*-DDD was subsequently identified as the principal toxic agent (Cueto and Brown, 1958). Mitotane apparently acts directly on the adrenal glands, producing degenerative lesions of the zona reticularis and the zona fasciculata in the cortex (Vilar and Tullner, 1959). The biochemical mechanism of its action is not known. Mitotane is administered orally.

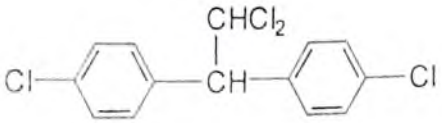
The drug is widely distributed in the body, but it apparently does not enter the cerebrospinal fluid (Moy, 1961). Like the insecticides DDT and DDD, a significant amount of the unaltered drug is stored in fat (Moy, 1961). On discontinuation of therapy, the drug disappears slowly from the serum over the course of several weeks.

Toxicity

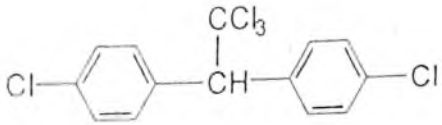
About 75 percent of patients receiving mitotane have some gastrointestinal side effects (Lubitz *et al.*, 1973). Side effects seen in the central nervous system, include lethargy and somnolence (40 per cent); dizziness or vertigo (17 per cent); weakness (21 per cent); and rarely, headache, confusion, tremors, visual distrubances, and retinopathy (Lubitz *et al.*, 1973). Rashes and changes in skin pigmentation occur in 13 per cent of patients (Lubitz *et al.*, 1973). Since adrenal suppression is the principal action of the drug, it should be temporarily discontinued following shock or severe trauma and because the depressed adrenal may not be able to rapidly secrete steriods, exogenous glucocorticoid should be administered.



Mitotane (o,p'-DDD)



DDD



DDT

Procarbazine

Procarbazine [1-methyl-2-*p*-(isopropylcarbamoyl) benzylhydrazine hydrochloride] was shown to be active against a variety of animal tumors (Bollag and Grunberg, 1963) and it now has an established role in the treatment of cancer in man. The biological effects and pharmacology of procarbazine have been reviewed by Reed in 1975 and its clinical application by Spivak in 1974.

Although procarbazine has been shown to have a number of biochemical effects, its mechanism of action is not yet clearly defined. The drug prolongs interphase and produces chromosome breaks in Ehrlich ascites tumor cells (Rutishauser and Bolag, 1963). Strand scission occurs when procarbazine is incubated with DNA in the presence of oxygen (Bernies *et al.*, 1963). If oxygen is replaced by an inert gas, or if peroxidase, or catalase is added, the viscosity of the DNA does not change. The parent drug undergoes auto-oxidation at 37°C in aqueous solution, producing hydrogen peroxide, which can degrade DNA.

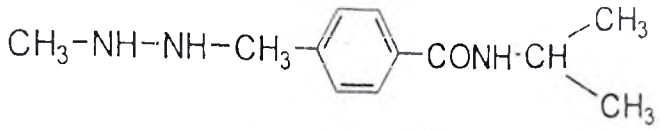
Use

Procarbazine is used in combination drug therapy of patients with advanced Hodgkin's disease as part of the MOPP [mechlorethamine, vincristine (Oncovin), procarbazine, prednisone] (De Vita *et al.*, 1972) and CVPP (cyclophosphamide, vinblastine, procarbazine, prednisone)

(Bloomfield *et al.*, 1976) regimens. It is also used in various drug protocols to treat non-Hodgkin's lymphomas (Spivack, 1974), small cell carcinomas of the lung (Nixon *et al.*, 1975) and malignant melanoma (Comis and Carter, 1974). Because of its activity against the intracerebral L1210 rat leukemia model and its good penetration into the cerebrospinal fluid, procarbazine has been used to treat malignant brain tumors (Cutin *et al.*, 1975).

Toxicity

The major toxicity of procarbazine is a dose-related, reversible bone marrow depression, with leukopenia and thrombocytopenia (Spvack, 1974). Nausea and vomiting occur frequently after the initial administration of the drug but tend to subside as therapy continues (Spivack, 1974). Procarbazine is also neurotoxic, and may produce altered levels of consciousness or peripheral neuropathy (seed Table 9-3) (Weiss *et al.*, 1976). Central nervous system depression ranges from mild drowsiness to profound stupor, and transient mental changes, including hallucinations, agitation and manic psychosis have also been reported (Weiss *et al.*, 1976) though they are rare. Paresthesias of the extremities and hypoactive deep tendon reflexes can occur; they are reversible on cessation of therapy. Procarbazine lowers plasma pyridoxal phosphate levels in animals and it has been suggested that this may play a role in its neurotoxic effect (Chabner *et al.*, 1969). Administration of pyridoxine, however, has not been found to reverse this toxicity in man.



Procarbazine

Cis-diamminedichloroplatinum(II)

Cis- diamminedichloroplatinum(II) (DDP) is one of a number of platinum coordination complexes with antitumor activity.

Several chemical requirements for the antitumor activity of platinum(II) complexes have been established. Since all the *trans*-compounds tested have been ineffective, the *cis*-configuration appears to be required.

Cis- diamminedichloroplatinum(II) appears to kill cells in all stages of the cell cycle (Drewinko and Gottlieb, 1975). The drug produces a selective and persistent inhibition of DNA synthesis in a variety of cell types, including phytohemagglutininstimulated human lymphocytes (Howle *et al.*, 1971) human amnion cell (Harder and Rosenberg, 1970) and Ehrlich ascites tumor cells (Howle and Gale, 1970).

Several studies of the association of DDP with both natural DNAs and synthetic polynucleotides show that the drug binds to guanine preferentially (Murchausen and Rahn, 1975) and also to adenine and cytosine. Multiple sites on the bases can be attacked but some differences between the reactions of *cis*- and *trans*-isomers may be found. The *cis*-isomer, for example, reacts with both the O^6 and N^7 of DNA guanine, whereas the *trans*-isomer

apparently does not interact with the O^6 guanine site (Millard *et al.*, 1975). There is considerable evidence that DDP exerts its cytotoxic effect through binding to DNA. The critical interactions with DNA have not yet been identified, and the difference between the cytotoxicity produced by the *cis*- and *trans*-isomers has not been adequately explained at either the molecular or the cellular level.

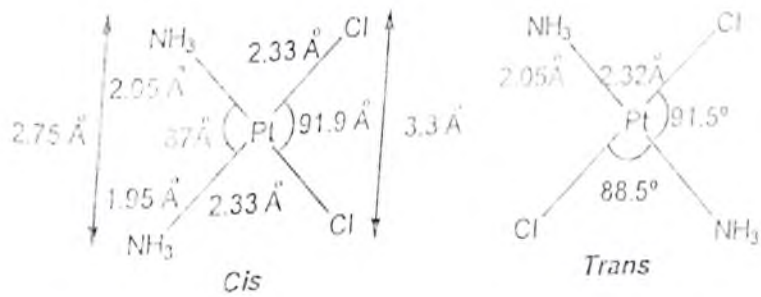
Use

Cis-diamminedichloroplatinum(II) is one of the most active drugs against testicular tumors and in combination with vinblastine and bleomycin, it produces complete remission in 74 per cent of patients with disseminated disease and partial remission in 26 per cent.[87] The drug produces therapeutic responses in about 25 per cent of patients with advanced ovarian adenocarcinoma who have failed to respond to, or have relapsed after the treatment with alkylating agents (Wiltshaw and Kroner, 1976). One of the more active drugs in the treatment of bladder cancer (Yagoda, 1977) DDP is also active against epidermoid carcinomas of the head and neck (Wittes *et al.*, 1977) and its role in the possible treatment of other types of cancer is being evaluated (Rozencwerg *et al.*, 1977). It is not known what factors determine whether a tumor will respond to DDP therapy, and the mechanisms of acquired resistance have not been identified.

Toxicity

Nausea and vomiting occur in virtually all patients receiving DDP, within 1 hour after drug administration, and last from 4 to 6 hours (and occasionally up to a week in especially sensitive patients) (Rozencweig *et al.*, 1977). The major dose-limiting effect is nephrotoxicity. Pathological changes in the kidney consist of focal acute necrosis, primarily affecting the distal convoluted tubules and collecting ducts, dilation of the convoluted tubules and formation of casts (Vitale *et al.*, 1977). The drug produces a dose-dependent ototoxicity that may be manifested by tinnitus or hearing loss or both (Piel *et al.*, 1974).

Although DDP products myelosuppression, the degree of leukocytopenia and thrombocytopenia is usually moderate (Rozencweig *et al.*, 1977). There have been several reports of patients experiencing anaphylactic types of reactions to DDP (Rozencweig *et al.*, 1977). Skin tests with DDP analogs showed that neither the chloride nor the amine groups in DDP were essential for reactivity but in this atopic hypersensitivity, there was no cross-reaction with three other platinum complexes of known antitumor activity (Khan *et al.*, 1975). In addition to acting as a hapten and binding to proteins to induce allergic reactions, DDP itself is immunosuppressive (Khan *et al.*, 1975).



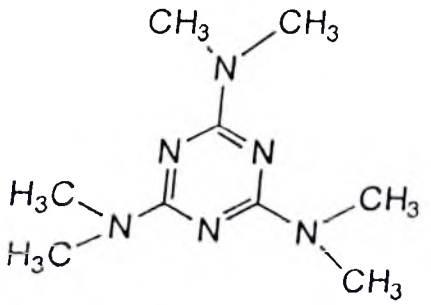
Hexamethylmelamine

Hexamethylmelamine (HMM), an investigational drug has been in clinical trial, for more than 10 years. Its structure is very similar to that of the alkylating agent triethylenemelamine. Although HMM has been shown to inhibit the incorporation of precursors into DNA and RNA by Ehrlich aseites tumor cells *in vitro* (Heere and Donnelly, 1971) the biochemical effects have not been studied in any detail and the mechanism of HMM action remains unknown.

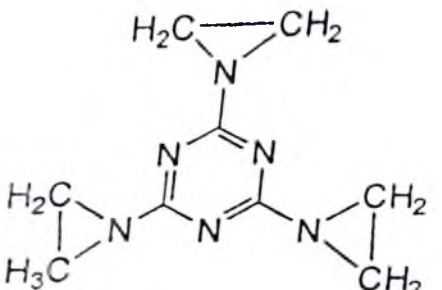
The drug has fairly wide spectrum of action against solid tumors (Lgegha *et al.*, 1976). It has established activity in cancer of the ovary and there is evidence for activity in cancer of the cervix and possibly uterine cancer (Devita *et al.*, 1976). It also possesses some activity against lung cancer (particularly the small cell type), lymphomas (both Hodgkin's and non-Hodgkin's types), and breast carcinomas (Lgegha *et al.*, 1976).

Toxicity

Anorexia, nausea, and vomiting are common side effects, being reported in 50 to 70 per cent of patients (Lgegha *et al.*, 1976). The nausea and vomiting appear to be related to an effect on the central nervous system rather than to local irritation of the gastrointestinal tract and they are often dose limiting (Lgegha *et al.*, 1976). Patients may occasionally experience abdominal cramps and diarrhea. After prolonged administration of HMM, a few patients experience a reversible neurotoxicity characterized by paresthesias, hyporeflexia and muscle weakness (Bergevin *et al.*, 1973). Ataxia and a Parkinson-like syndrome have also been reported (Bergevin *et al.*, 1973). The mechanism of the neurotoxicity its unknown but pyridoxine has been administered in an attempt to ameliorate it (Lgegha *et al.*, 1976). Some patients may also have a central nervous system involvement, with depression confusion, and agitation. Rarely, pruritis and skin rash occur (Lgegha *et al.*, 1976).



Hexamethylmelamine



Razoxane

Razoxane [1, 2-di(3,5-dioxopiperazin-1-yl)propane] (ICRF 159) is one of the groups of *bis*-dioxopiperazines developed at the Imperial Cancer Research Fund Laboratory (ICRF). These compounds, analogs of the chelating agent ethylenediaminetetraacetic acid (EDTA), were synthesized with the rationale that they might be activated after entry into the cell. Razoxane has a fairly wide spectrum of antitumor activity in animal systems but its action does not appear to depend on chelation. The studies on the biological effects and pharmacology of razoxane have been reviewed (Bakowski, 1976). The drug is undergoing clinical trial.

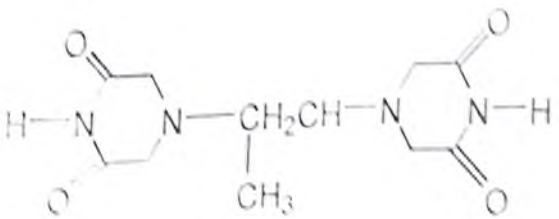
Very little is known about the biochemical action of razoxane. It has been reported that the drug kills cells only during a brief period of the generation cylce (Hellmann and Field, 1970). Recent experiments with lectin-stimulated human lymphocytes suggest that progression into late mitosis is blocked only when the cells are exposed to the drug during the premitotic and early mitotic (G_2 M) phases of growth (Sharpe *et al.*, 1970). Even though cell division is inhibited, RNA synthesis continues and multinucleate cells accumulate in cultures exposed to low concentrations (10 *ng*/ml) of razoxane (Hallowes *et al.*, 1974). Since plateau phase cells are much less sensitive to killing than cells in exponential growth, the cytotoxicity is clearly proliferation dependent (Taylor and Bleehen, 1977) but strict G_1 phase specificity of the drug action has not been unequivocally established.

Uses

Razoxane has been found to have some activity in acute leukemia, non-Hodgkin's lymphomas, and colorectal carcinoma (Bellet *et al.*, 1977). Although drug-resistant cell can be selected in culture, they are not cross-resistant with a variety of other anticancer agents (except for a small cross-resistance with anthracycline antibiotics) (White and Creighton, 1976) and cross-resistance has not been apparent in clinical studies (Bakowski, 1976).

Toxicity

The major adverse effect is bone marrow depression, primarily a leukopenia that is dose limiting (Bakowski, 1976). Thrombocytopenia and anemia occur less often and are generally mild. Nausea and vomiting are experienced by 40 to 60 per cent of patients and alopecia is common (12 per cent), becoming especially severe with multiple courses of therapy (Bellet *et al.*, 1977). Oral mucositis has been reported rarely, as have dermatitis and a flu-like syndrome. Razoxane has radiosensitizing, immunosuppressive (primarily B-cell function), and mutagenic activity (Taylor and Bleehen, 1977).



Razoxane
(ICRF 159)

12. MOLECULAR APPROACHES TO DIAGNOSIS OF CANCER

Many cancers can be controlled with existing methods of therapy, provided that they a treated early enough. These cancers include the major killers, cancers of the lung, breast an gastrointestinal tract. Methods for early detection of cancers are therefore of great benef to the patient and, while not providing a cure in themselves, make the patient more curable

The two main areas of early detection are a general population screening for the presence o tumour and screening of patients at high risk. This includes the monitoring of patients who have been apparently successfully treated for cancer to detect recurrence and thereby to initiate further therapy.

The ideal tumour marker could be used as a screen for the general population and would detect only those who had cancer even in its earliest stages. These patients would be further investigated, treated and hopefully cured. Unfortunately, no such test exists, although many claims to early cancer tests have been made. Such screening is fraught with difficulty and some of the problems have been outlined by Bagshaw.[1] Nevertheless, some population screens for patients at risk are felt to be useful. The Pap test for cervical cancer in woman, the stool Guaiac test for the detection for occult blood from colorectal cancer and the education of the public to the signs and symptoms of cancer should all show their effects in decreased death rates or prolonged survival times. The yearly biochemical blood or urine test for cancer, however, still eludes us.

Detection of tumour recurrence

It is in the area of detecting residual tumour after surgery or therapy and in the earlier detection of tumour recurrence that biochemical markers make their greatest impact. These markers can serve to give the oncologist information about prognosis and the effectiveness of therapy. Probably the nearest to the ideal tumour marker available is human chorionic gonadotropin (HCG) used to monitor gestational cancers. a-Fetoprotein is another marker routinely used to monitor hepatoma and certain gestational cancers. The carcinoembryonic antigen (CEA) is now the most widely used for the tumour markers though not always behaving ideally, still could be used for monitoring a range of the most common cancer. On the other side acid phosphatase is used for the investigation of cancer of the prostate.

Limits of tumour detection

Physical methods

These are usually classified under radiology and include X-ray, computerised tomography (CT scan), nuclear magnetic resonance (NMR scans) and various isotopic methods that include liverspleen scan, bone scans, etc. In general the best resolution obtainable under ideal conditions is the detection of a tumour between 0.5 and 1 cm in diameter. This represents about 1 g of tissue or 10^{12} tumour cells. Advances in physical methods of detection occur all the time but it is unlikely that these limits of detection will be significantly improved on in the foreseeable future.

Biochemical methods

A biochemical test which includes immunochemical assay procedures, should be the most sensitive way to detect the presence of a tumour. If a cancer produces a unique substance and this substance finds its way to urine or blood, and a test sensitive enough to detect nanogram quantities or less is available, then it would be theoretically possible to detect the presence of a single tumour cell. Tests such as those for HCG or CEA in plasma are capable of detecting small amounts of tumour, but many factors influence the levels of tumour markers.

Ideal tumour marker

This would be a molecular substance produced by all tumour cells that distinguishes them from normal cells. Its production must be directly related to tumour mass and it must be found in sera or urine allowing a rest suitable for automation to be produced. The test would detect cancer reliably and early enough for curative therapy.

Biochemical methods for mancer detection (table 3)

The biochemical monitoring of cancer has become a practical proposition since the development of highly specific methods for measuring substances in biological fluids. The major advancement was the development of the radioimmunoassay (RIA) and later the enzyme-linked immunoassay (EIA) methods. These procedures are capable of quantitatively detecting down to picogram (10^{-9}g) per millilitre amounts of substances in biological fluids provided that specific antibodies are available. The RIA relies on the competition for binding to the antibody between the purified substance that has been radiolabelled (in the case of proteins such as HCG or CEA with ^{125}I) and the substance in the biological fluid. Unbound radiolabel is separated from bound radiolabel and the amount of bound radioactivity is inversely proportional to the amount of material in the sample. The EIA works slightly differently. The antibody is bound to a solid support (e.g. a nylon bead) and is incubated with the sample. Any antigen present binds to the antibody. A second antibody conjugated to an enzyme (often peroxidase) is incubated with the bead and reacts with the bound antigen. Incubation of the bead with a chromogenic substrate for the enzyme results in colour development which is proportional to the amount of antigen in the sample. A related procedure used in tumour diagnosis is immunohistochemical staining of tissue sections to detect specific antigens. This involves incubating a paraffin-embedded tissue section with a specific antibody followed by incubation with an anti-antibody conjugated to an enzyme (again often peroxidase) and then the section is incubated with a substrate that gives an insoluble coloured product. The deposition of the product on the section indicates the presence of the antigen.

Table 3 : Some biochemical tests in clinical use for detection and monitoring of cancer

Substance (in serum)	Structure	Mol. Wt. assay	Method of	Use
Human chorionic gonadotropin (HGG)	Glycoprotein	46000 subunit 16000 subunit 30000	RIA, EIA	Gestational cancers

Carcinoembyonic antigen (CEA)	Glycoprotein	180000	RIA, EIA	Wide range, including cancer of colon, breast, lung, pancreas and ovary
a-Fetoprotein (AFP)	Glycoprotein	70000	RIA, EIA	Hepatocellular, testicular cancers
Acid phosphatase	Glycoprotein	102000	Spectrophotometric	Prostatic cancer
Calcitonin	Peptide	3500	RIA, EIA RIA	Medullary cancer of the thyroid, breast cancer?
b2-Microglobulin	Protein	11800	RIA, EIA	Lymphoma, multiple myeloma

Radioimmunolocalisation of cancer

A more recent development in the detection of cancer has been to use radiolabelled antibodies against tumour-associated antigens to localise4 tumours within the patient. The method involves injection of radiolabelled (^{131}I) antibody and a search for the tumour by using external scintiscanning. The first successful studies were carried out in humans using antibodies to CEA by Goldenberg and his colleagues (Goldenberg *et al.*, 1978). varying success with these methods has been reported but generally both primary and secondary tumours can be visualised provided that they are larger than 1-2 cm in cm diameter. The presence of even large amounts of antigen in the circulation seems to make little difference to the success of the method. The main emphasis with radioimmunolocalisation has been to use carcinoembryonic antigen (CEA), a-Fetoprotein (AFP) or Human chorionic gonadotropin (HCG) as the target antigens. Other targets are now being studied and the use of monoclonal antibodies should result in an expansion of these studies. The few investigations with monoclonal antibodies to CEA in patients have given similar results totargeting with polyclonal antisera. However, studies with monoclonals in experimental animal systems has shown some advantages over the polyclonal antisera, including better tumour to normal tissue ratios of radioactivity. A great deal of effort is also being expended to improve the resolution of procedure and a number of approaches are being used. Changing the radioactive isotope from ^{131}I to ^{123}I because of its better dosimetry, or ^{111}I because of its suitability for detection by conventional gamma-cameras and intracellular accumulation, may give better resolution. Improvements are being made in the scanning techniques and in methods for background radioactivity subtraction.

13. THE FUTURE

The examples of clinically useful tests for cancer described above demonstrate the lack of specificity in cancer detection. However, these tests used properly are useful and if specificity were the sole criterion there would be no tests for cancer. No doubt in the future the search for specific tests will continue and with the expanding use of monoclonal antibodies and the use of recombinant DNA technology the chances of finding tumour-specific molecules are better than ever before.

The recent discovery of oncogenes and their products should cause a great expansion in the effort to determine if these proteins can be used for early detection of cancer or for diagnosis of premalignant states. More effective antibodies to new markers, possibly membranebound, should improve radioimmunolocalisation. Similarly the use of human monoclonal antibodies in place of the mouse monoclonals now in general use should reduce the problems of immune responses to the injected antibodies. Research on the presently available markers such as CEA will also continue with the aim of improving their use. Studies of the factors affecting their plasma concentrations could lead to ways of increasing their levels in blood perhaps by blocking their metabolism. These studies could lead to earlier detection of recurrence. Research in cancer detection has expanded greatly over the past ten years and should continue to expand. Further advances in this area should have a substantial effect on survival rates for many of the common cancers.

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किसी जिज्ञासा को शांत करना, एवं ज्ञान प्राप्ति करके।
सामाजिक समस्याओं का निराकरण करना, तथा घटना का यथार्थ चित्रण ही शोध है।।

—डॉ० अशोक कुमार यादव

लेखक परिचय


डॉ० अशोक कुमार यादव

डॉ० अशोक कुमार यादव का जन्म, ग्राम-भिलकपुर, तहसील-बहरोड़, जिला- अलवर (राज०) में हुआ है। आपने राजस्थान विश्वविद्यालय से "एम०एससी० (रसायन विज्ञान), एम०एड०, एम०ए० (समाजशास्त्र), एलएल० बी०" की उपाधि प्राप्त की है, सन् 2011 में "बाल अपराध में परिवार, पुलिस एवं कानून की भूमिका : जयपुर शहर का एक समाजशास्त्रीय अध्ययन" विषय पर राजस्थान विश्वविद्यालय से समाजशास्त्र विषय में, "पीएच०डी०" की उपाधि प्राप्त की, तथा यू०जी०सी० से "नेट परीक्षा", और आर० पी० एस० सी० की "सैट (स्लेट) परीक्षा", समाजशास्त्र विषय में पास की। आप कॉलेज स्तर पर बी०ए० और एम०ए० कक्षाओं में, समाजशास्त्र विषय के अध्यापन में कार्यरत रहे हैं, तथा बी०एड० एवं एम०एड० कक्षाओं में भी अध्यापन कार्य किया है। आप एन०सी०सी० के बेस्ट कैडेट, तथा आर०एस०एस० के सक्रिय कार्यकर्ता भी रहे हैं। आप विभिन्न सामाजिक एवं शैक्षणिक संस्थाओं में भी प्रत्यक्ष एवं परोक्ष रूप से अपनी सेवाएँ प्रदान करते रहे हैं।

आपके नारी-शक्ति, बाल-अपराध, भूमण्डलीकरण, समाज, इक्कीसवीं सदी में अध्यापक की भूमिका, अच्छा समाज, नारी की स्थिति, भारत में समस्याएँ, मानवाधिकार, पुलिस की भूमिका, निर्धनता की समस्याएँ, महिला आरक्षण, ग्रामीण महिला, महिला अपराध, बाल-अपराध और परिवार, बाल-अपराध में पुलिस एवं कानून की भूमिका, गाँधीवादी विचारधारा, गाँधीदर्शन की विवेचना, महिलाओं पर होने वाले अपराध, हरिजन पर गाँधी विचार, परिवार की भूमिका और समाज, परिवार विकास, कानून व्यवस्था और महिला शक्ति, स्वामी विवेकानन्द की प्रेरक जीवनी, हिन्दू विवाह एवं मानसिक विकार, जैन धर्म एवं विवाह, हिन्दू विवाह और दहेज, विवाह एक संस्थान एवं समाज में शिक्षा आदि विषयों पर नेशनल और इन्टरनेशनल लेख प्रकाशित हुए हैं। आपने "वर्तमान समय में बढ़ती बाल अपराधी प्रवृत्ति : पुलिस एवं कानून की भूमिका", "बाल अपराध एक विकट समस्या : परिवार की अहम् भूमिका", "समाजशास्त्रीय विचार", "समाज में महिलाओं की भूमिका", "महात्मा गाँधी के विचार", "स्वामी विवेकानन्द : एक अनमोल रत्न", "पीड़ित महिला एवं समाज", "बाल अपराध : एक सामाजिक समस्या", "परिवार एवं समाज", "समाज में विवाह", "आजादी के दिवाने", "सामाजिक समस्याएँ", "स्वामी विवेकानन्द : शिक्षा का आदर्श" तथा "महात्मा गाँधी : शिक्षा एवं दर्शन" आदि विषयों पर पुस्तकों का लेखन कार्य भी किया है। आपने विभिन्न लेखकों की लगभग 25 पुस्तकों में लेख भी प्रस्तुत किये हैं, तथा लगभग 40 इन्टरनेशनल एवं नेशनल सेमिनार/कॉन्फ्रेंस में पेपर भी प्रस्तुत किये हैं। आप "एन इन्टरनेशनल जरनल ऑफ रिसर्च एण्ड डवलपमेण्ट इन न्यू एरा" के "मुख्य सम्पादक" पद पर कार्यरत हैं। वर्तमान में आप "कॉलेज में प्राचार्य पद" पर कार्यरत हैं, तथा विभिन्न विश्वविद्यालयों में शोध निर्देशक के रूप में कार्यरत हैं। आपके राजस्थान पत्रिका, और दैनिक भास्कर में लेख आते रहते हैं। आपकी पाठ्य शैली व अध्यापन शैली अत्यन्त सुग्राही है।

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शोध पद्धतियाँ
डॉ० अशोक कुमार यादव
ASSOCIATED

शोध पद्धतियाँ
RESEARCH METHODOLOGY

उत्तर खोजने की क्रमबद्ध एवं व्यवस्थित प्रणाली ही, शोध पद्धतियाँ है।
—डॉ० अशोक कुमार यादव

डॉ० अशोक कुमार यादव

पुस्तक-परिचय

मनुष्य एक जिज्ञासु प्राणी है, उसकी आंतरिक जिज्ञासा, सदा से ही, अनुसंधान का कारण बनती रही है। अनुसंधान, खोज, गवेषणा, अन्वेषण एवं शोध पर्यायवाची है। शोध में उपलब्ध विषय के तथ्यों में, विद्यमान सत्य को नवस्थापित कर, पुनर्स्थापित किया जाता है। शोध में शोधार्थी के सामने तथ्य मौजूद होते है, उसे उसमें से अपनी सूझ व ज्ञान द्वारा, नवीन सिद्धियों को उद्घाटित करना होता है। जब सामाजिक क्षेत्र में प्रश्नों के उत्तर खोजने का क्रमबद्ध एवं व्यवस्थित प्रयास किया जाता है, तो उसे ही "सामाजिक अनुसंधान" कहते है। व्यापक अर्थ में अनुसन्धान (Research) किसी भी क्षेत्र में "ज्ञान की खोज करना" या "विधिवत गवेषणा" करना होता है।

वैज्ञानिक अनुसन्धान में, वैज्ञानिक विधि का सहारा लेते हुए, जिज्ञासा का समाधान करने की, कोशिश की जाती है। नवीन वस्तुओं की खोज, और पुराने वस्तुओं एवं सिद्धान्तों का पुनः परीक्षण करना, जिससे कि नए तथ्य प्राप्त हो सकें, उसे शोध कहते है। शोध उस प्रक्रिया अथवा कार्य का नाम है, जिसमें बोधपूर्वक प्रयत्न से, तथ्यों का संकलन कर, "सूक्ष्मग्राही एवं विवेचक बुद्धि" से, उसका अवलोकन-विश्लेषण करके, नए तथ्यों या सिद्धांतों का उद्घाटन किया जाता है।

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डॉ० अशोक कुमार यादव

डॉ० अशोक कुमार यादव का जन्म, ग्राम-मिलकपुर, तहसील-बहरोड, जिला- अलवर (राज०) में हुआ है। आपने राजस्थान विश्वविद्यालय से "एम०एससी० (रसायन विज्ञान), एम०एड०, एम०ए० (समाजशास्त्र), एलएल० बी०" की उपाधि प्राप्त की है, सन् 2011 में "बाल अपराध में परिवार, पुलिस एवं कानून की भूमिका : जयपुर शहर का एक समाजशास्त्रीय अध्ययन" विषय पर राजस्थान विश्वविद्यालय से समाजशास्त्र विषय में, "पीएच०डी०" की उपाधि प्राप्त की, तथा यू०जी०सी० से "नेट परीक्षा", और आर० पी० एस० सी० की "सैट (स्लेट) परीक्षा", समाजशास्त्र विषय में पास की। आप कॉलेज स्तर पर बी०ए० और एम०ए० कक्षाओं में, समाजशास्त्र विषय के अध्यापन में कार्यरत रहे हैं, तथा बी०एड० एवं एम०एड० कक्षाओं में भी अध्यापन कार्य किया है। आप एन०सी०सी० के बैस्ट कैडिट, तथा आर०एम०एस० के सक्रिय कार्यकर्त्ता भी रहे हैं। आप विभिन्न सामाजिक एवं शैक्षणिक संस्थाओं में भी प्रत्यक्ष एवं परोक्ष रूप से अपनी सेवाएँ प्रदान करते रहे हैं।

आपके नारी-शक्ति, बाल-अपराध, भूमण्डलीकरण, समाज, इक्कीसवीं सदी में अध्यापक की भूमिका, अच्छा समाज, नारी की स्थिति, भारत में समस्याएँ, मानवाधिकार, पुलिस की भूमिका, निजीकरण की समस्याएँ, महिला आरक्षण, ग्रामीण महिला, महिला अपराध, बाल-अपराध और परिवार, बाल-अपराध में पुलिस एवं कानून की भूमिका, गाँधीवादी विचारधारा, गाँधीदर्शन की विवेचना, महिलाओं पर होने वाले अपराध, हरिजन पर गाँधी विचार, परिवार की भूमिका और समाज, परिवार विकास, कानून व्यवस्था और महिला शक्ति, स्वामी विवेकानन्द की प्रेरक जीवनी, हिन्दु विवाह एवं मानसिक विकार, जैन धर्म एवं विवाह, हिन्दु विवाह और दहेज, विवाह एक संस्थान एवं समाज में शिक्षा आदि विषयों पर नेशनल और इन्टरनेशनल लेख प्रकाशित हुए हैं। आपने "वर्तमान समय में बढ़ती बाल अपराधी प्रवृत्ति : पुलिस एवं कानून की भूमिका", "बाल अपराध एक विकट समस्या : परिवार की अहम् भूमिका", "समाजशास्त्रीय विचार", "समाज में महिलाओं की भूमिका", "महात्मा गाँधी के विचार", "स्वामी विवेकानन्द : एक अनमोल रत्न", "पीड़ित महिला एवं समाज", "बाल अपराध : एक सामाजिक समस्या", "परिवार एवं समाज", "समाज में विवाह", "आजादी के दिवाने", "सामाजिक समस्याएँ", "स्वामी विवेकानन्द : शिक्षा का आदर्श" तथा "महात्मा गाँधी : शिक्षा एवं दर्शन" आदि विषयों पर पुस्तकों का लेखन कार्य भी किया है। आपने विभिन्न लेखकों की लगभग 25 पुस्तकों में लेख भी प्रस्तुत किये हैं, तथा लगभग 40 इन्टरनेशनल एवं नेशनल सेमिनार/कॉन्फ्रेंस में पेपर भी प्रस्तुत किये हैं। आप "एन इन्टरनेशनल जरनल ऑफ रिसर्च एण्ड डवलपमेण्ट इन न्यू एरा" के "मुख्य सम्पादक" पद पर कार्यरत हैं। वर्तमान में आप "कॉलेज में प्राचार्य पद" पर कार्यरत हैं, तथा विभिन्न विश्वविद्यालयों में शोध निर्देशक के रूप में कार्यरत हैं। आपके राजस्थान पत्रिका, और दैनिक भास्कर में लेख आते रहते हैं। आपकी पाठन शैली व अध्यापन शैली अत्यन्त सुग्राही है।

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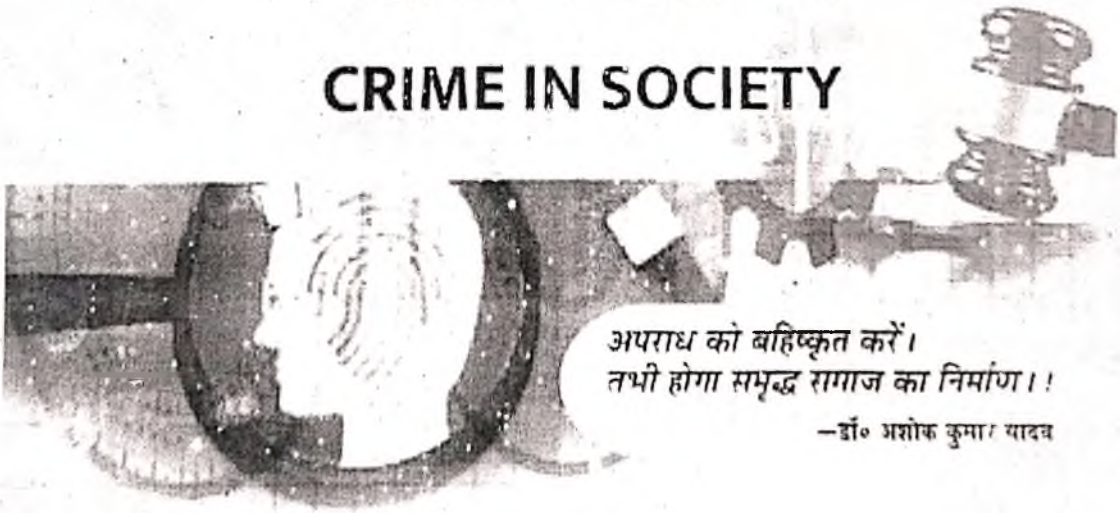
समाज में अपराध

CRIME IN SOCIETY



अपराध को बहिष्कृत करें।
तभी होगा समृद्ध समाज का निर्माण।।

—डॉ० अशोक कुमार यादव

डॉ० अशोक कुमार यादव

Chapter-10

Crime Against Older Adults

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Abstract

Although often perceived to be a problem of the young, violence commonly affects older adults, a rapidly growing segment of the population. Violence can be directed toward older adults, self-directed, or perpetrated by older adults against others. Across forms of violence, firearm access increases lethality, and veterans may be a particularly high-risk population. The forms of violence in older adults have some common risk factors and common challenges for prevention. The integration of prevention strategies across the life span, disciplines, and types of violence offers promise for promoting older adult health and well-being.

Looking forward, key areas for attention will include raising awareness about these topics and prioritizing funding for the implementation and evaluation of violence prevention interventions in health care settings and the community. Violence is often perceived to be a problem of the young, but it persists into later life and has a major impact on older adults. The anticipated explosive growth of the US population ages sixty five and older, which

expected to double to eighty three million by 2050, will increase the urgency of addressing this problem. Unfortunately, violence in older adults has received little attention to date from researchers, policy makers, or providers in the fields of social services, medicine, or criminal justice. Violence takes three main forms in older adults, violence directed toward older adults, self directed violence, and violence perpetrated by older adults against others. In this article, we summarize the available data on each of these forms of violence, including risk factors and challenges as well as strategies for preventing and responding to violence.

Introduction

Although older adults report violent victimization at lower rates than other populations do, perpetrators may target older adults because of their perceived vulnerability or lower likelihood of reporting. While violence may be perpetrated by strangers, a substantial portion is physical or sexual elder abuse, in which the perpetrator is a known person in a position of trust. Based on law enforcement data, 50 percent of older victims of violent crime were assaulted by a family member. This violence includes abuse of a dependent older adult by a caregiver as well as incidents involving an independent older adult.

Violence toward Older Adults

IPV is also an important type of violence toward older adults, although it may also be categorized as elder abuse. As is the case with younger adults, it most commonly involves a female victim and a spouse or partner as the perpetrator. Though many older adult IPV victims have suffered for decades in abusive relationships, others experience it for the first time in later life. This can occur either from a new partner or from a long term partner in the context of cognitive impairment or stress related to care giving. In some cases, the abuse is mutual, with both the partner delivering care and the partner receiving care acting violently toward one another. Recent research from seven US states emphasizes the lethality of IPV among older adults 23 perrent of all women killed by their partners were ages fifty and older, and 31 percent of homicides among women ages sixty five and older were IPV related.

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