CHAPTER- V

DISCUSSION
Generally examining of a new drug suffers from many steps i.e. biological tests, biological standardization procedures, study of individual variation in drug response, dose response relations, therapeutic index, valuation of drug toxicity and other advance effects and clinical trials. All these procedures are complicated, time taking and expansive and thus require long term planned team work.

The development of a new drug also requires a number of biological standardization procedures before the drug comes for routine use in patients. The discovery of chemical compounds and their specific action is passing through complicated procedure lasting several years and involve thousands of tests and give a specific name. This involves a series of investigations called formulation research.

The essential aspects of formulation researches are stability, purity, base, compatibility, disintegration, solubility, pH, surface tension, viscosity, calorie value, melting points, flavour, sterility,
packing procedure; storage, quality control etc.

Thus, the evaluation and development of a new drug is in itself an independent discipline and requires the skill of a pharmacologist, pharmacist and many clinicians.

Generally the indigenous drug evaluation should pass through same succession of procedures as mentioned above, but it should be remembered that these drugs are not completely unknown substances. These substances are already known to some extent and in practice for their certain effects. Many of their toxic and side effects are known and their effective dose is also known. These existing information may be guideline in the re-evaluation of these drugs. The prevalent use and textual description about the drug may be helpful in determining the area of trials. The trial of the drug for a particular disease may be directly started in patients and if the preliminary trials are encouraging the drug may be shifted for laboratory studies, isolation of active principles and then the clinical trial of active principles may be undertaken. The direct clinical trial of such drug may not be objectionable-extent for their toxic effect and side effects and it is not unsafe to undertaken clinical trial without laboratory studies. Inspite of this, there are schools which prefer laboratory investigation to procede clinical trial is preferable.

The main object of present investigation is the study of the
identification and vegetation of the plant *Boerhaavia diffusa* Linn. (Punarnava); cause of reduction of vegetation of the medically valuable plants; cultivation of the medicinal plant including seed collection; germination; growth; development; extraction of root extract; and production of medically used alkaloid "punarnavine" in the form of root extract for the treatment of Urinary disorders by using fertilizer mixture (15:15:15) and plant hormones like NAA, IAA, GA₃ and their interactions.

In view of many controversies prevailing about the identity of Indian indigenous drugs, it is generally considered essential to go for a preliminary identification study and definition of a particular drug before undertaking the detailed testing procedures. The characteristic feature of plant *Boerhaavia diffusa* Linn. is already discussed earlier for identification.

**Vegetation:**

As regards the vegetation of Punarnava (*Boerhaavia diffusa* Linn.), it is found in many tropical and warm climate countries. It is indigenous to India where it is found in abundance in the Warmer parts of the country on West land, bank of the ponds, ditches, pastures, dam of the river, side of the canal and uncultivated lands. Commonly found spreading and trailing on the ground, open
pastures, fruit Orchards and even on the old buildings. The Punarnava plant flourishes well in rainy seasons, medium in early winter and summer but looks dull in late winter (December-January) and late summer (April, May and June) respectively.

At present, the places ie. waste land, pond side, ditches pastures dam of the river, side of the canal and railways which are the most suitable for the vegetation of this medicinal plant Punarnava (Boerhaavia diffusa Linn.) responsible for the control of Urinary disorders are going to be captures by bad elements of the society, causing reduction in area and vegetation of this plant all over the district. Thus, it is necessary to cultivate this medicinally valuable plant to meet the demand of the people to control urinary disorders and many other diseases.

To solve this problem, this experiment was conducted to increase the vegetation of the Boerhaavia diffusa Linn.

Nearly all land plants pass through a phase of dormancy at some stage in the life cycle, either as spores in the lower plants such as bryophytes and ferns or as seeds in the case of the higher plants.

generally, plant growth is a continuous process from germination to death. However, all most all plants experience sometime in their life cycle periods when growth is temporarily
suspended or at least retarded, to the point of not being visibly detectable. Interestingly enough, this situation may usually be observed in seeds and buds, plant parts associated with either the propagation of the plant or its continued development.

Seeds will not germinate under dry conditions, but will readily germinate if water is imbibed. A suspension of growth may also occur because of the concentration of some growth inhibitors, or it may be caused mechanically by the mere presence of a strong durable enclosing structure that does not allow for the expansion of growth. The presence of membranes or seed coats impermeable to water or oxygen may also keep growth in an arrested state. Finally, many seeds and buds requires special conditions of light, oxygen and temperature to proceed germination (Devlin, 1972).

In our experiment, no seed dormancy in *Boerhaavia diffusa* Linn. was recorded. When the matured and well dried seeds, collected from the living plants, were placed in water and solution of NAA, IAA and GA₃ for 12 hours and transferred to another Petridishes having wet blotting paper germination of seeds was pronounced.

The germination of many seeds is affected by light, such seeds are said to be photoblastic. As is well known, the most important factors commonly affecting the germinations of seeds are water, aeration (oxygen), temperature and light. The usual criterion
of germination is the emergence of the radicle through the seedcoats. In lettuce seeds, both cell-division and cell-elongation are taking place at 12-14 hours after sowing at 25°C, where radicle elongation can first be detected (Evenari et al., 1957). The promotion of seed germination by light was noted in 1860 and the inhibition in 1903 (Toole et al., 1956).

The present research work where the effects of different concentrations of NPK mixture (15:15:15), NAA, IAA, GA3 and their interaction on germination, flower initiation, flowering, fruiting, seed production on root development and alkaloid (Punarnavine) synthesis responsible for the treatment of urinary disorders have been studied, show that the germination of seeds is greatly improved with treatment of Naphthalene acetic acid (NAA). Indole acetic acid (IAA) and Gibberellic acid (GA). The seeds treated with these plants hormones not only increase the germination % but also increase the rate of germination ie early emergence of plumule and radicle in comparison to control and NPK mixture. Pandey and Sinha (1972) also reviewed the use of hormone in agriculture. Now a day, NAA and IAA are most widely used in soaking seeds for germination. Pre-sowing treatment of seeds of Phaseolus aureus with IAA and NAA resulted an increase in germination at low concentration and
Naphthalene acetic acid (NAA) is also reported to stimulate germination of *C. capsularis*, *C. olitoreus* and *Crotolarea juncea*. In our experiment, NAA was found more effective than IAA. Similar results were also observed in potato and Grand Rapid Lettuce by Wilkins (1969). In potato, the sensivity to IAA was never very great, and the best results were observed with NAA. Gibberellic acid (GA₃) was found more sensitive in increasing the development of plumule and radicle and germination % in comparison to other hormones like NAA and IAA as well as control. The accelerating effect of GA₃ on germination of seeds of *Morus indica* was also reported by Pandey and Sinha (1972). Salisbury and Ross (1986) also emphasized that gibberellins promote germination of dormant seeds.

Treatments of auxins (Naphthalene acetic acid and Indole acetic acid) and gibberellins (Gibberellic acid) in the present investigation were found to have promotory effects on growth of axis of embryo (early development of radicle and plumule) and growth of plant of *Boerhaavia diffusa* Linn. as reported by Singh (1998).

**Growth:**

The treatment of Naphthalene acetic acid, Indole acetic acid,
Gibberellic acid and NPK mixture were also found to increase the height, node number, vegetative branches, fresh weight of shoot as well as fresh weight of most valuable part of the plant i.e. root but the treatment of Naphthalene acetic acid was found most effective in increasing the root growth than other hormones. Also in this research work, the interaction of NPK + NAA, NPK + IAA, and NPK + GA₃ were found to give better results in respect of plumule and radicle development, height, branches, leaves, fresh weight of shoot and root respectively in comparison to hormones applied alone (without NPK mixture). Wetmore et al., (1951), Steward et al., (1956) and Heslop-Harrison (1967) have also shown that growth and differentiation of organs in plant is the result of interaction of growth regulating substances such as auxins, gibberllins and Kinins. Thimann and Went (1934) showed that Indole Acetic acid stimulate root initiation from stem cuttings. As first shown in 1930s auxins will promote elonation of sections, excised from roots or even intact roots of many species (Scott, 1972; Batra et al., 1975), but only at extremely low concentrations, depending on the species and age of roots. At higher concentrations, elongation is almost always inhibited. Synthetic auxins such as Naphthalene acetic acid and Indole butyric acid are usually more effective than Indole acetic acid.
Pandey and Sinha (1972) also reviewed the effect of NAA on mango varieties and noted that 10% NAA induced 100% rooting in Desehri while 20% in Langera. They also reviewed that with higher concentration, not only root initiation is inhibited, the stimulation of rooting caused by auxins is also counteracted. The application of relatively higher concentration of IAA to roots not only retards root elongation but causes a noticeable increase in the number of branch roots. The higher concentration of NAA and IAA in our experiment, was also found to retard the root development in *Boerhaavia diffusa* Linn. Evans and Ray (1969) also obtained an increase in corn and oat coleoptile elongation-with lower concentration and vice-versa with higher concentration.

More recently, it has been found that the action of auxin in roots is similar to that in stem, but that the concentration of auxin stimulatory to stem growth are inhibitory to root growth. In other words, roots are much more sensitive to auxin than stems and real stimulation of root elongation may be achieved if low enough concentration are used (Jackson, 1960; Devlin and Jackson, 1061). Similar results were also obtained by Pengelly and Torrey (1982), Mulkey *et al.*, (1982) and Wieler (1984) respectively.

In our investigation, the application of Gibberellic acid (GA$_3$) was found to increase the elongation of radicle and plumule,
internodes, petiole, and stem; increase in the height of plant etc. Pharis and Kuo (1977) also reported that gibberellins have the unique growth of many intact plants. Most dicots and some monocots respond by growing faster when treated with gibberellins. Cabbage and other species in the rosette form that have short internodes some times grow 2 metres tall and then flower after GA₃ application. Short bush beans become climbing pole beans and genetic dwarf mutants of rice, maize, peas, watermelons, squash and cucumber exhibit phenotypically tall characteristics of normal varieties when treated with GA₃ (Salisbury and Ross, 1986). Similar results were also obtained in maize, rice and pea (Phinney and Spray, 1982). The work of several workers as reviewed by Black and Edelman (1970) on gibberellic acid show that the application of gibberellic acid to the leaf-blade or in the axil, Cabbage grow several feet tall and lettuce become vine like. Many dwarfs, for example, dwarf maize and peas treated with gibberellin have a long stem. In the cereals i.e. maize and rice, internodal growth extension of leaf sheath and blade are all promoted. GA₃ was found to promote root development. Similar results were also obtained in jute and tomato.
Flowering:

In this experiment, the application of Naphthalene acetic acid, Indole acetic acid, and Gibberellic acid were found to have promotery effect in flower initiation and flowering of *Boerhaavia diffusa* Linn. No earliness in flowering was recorded in plants treated with NPK mixture significantly. In all these treatments, Gibberellic acid was found more effective in initiation of flowering and flowers/plant than NAA and IAA. The higher dose of these hormones were found to have adverse effect on flowering.

The combined effect (interaction effect) of NPK mixture and hormones was found superior to hormones i.e. NAA, IAA and GA₃ applied alone in respect of flower initiation and flowers/plant.

The transformation of a vegetative shoot into reproductive shoot has attracted the attention of botanists since the middle of the 19th century. The idea that there are organ forming substances in plants was first given by Julius Sachs in 1880. Garner and Allard's discovery of photoperiodism in plants in 1920 opened a new era of research on physiology of flowering and the work since then has been extensively reviewed (Lang, 1952, 1965; Doorenbos, Wellensick 1959; Salisbury, 1961; Hillman, 1964; Searle 1965; Chailakhyan, 1968 and Kolli, 1969).

Flowering is an important stage in a plant's life because the
transition from vegetative to generative development involves essential changes in metabolism, translocation of nutrients and arouses essential formative processes which are connected with the development of organs of sexual reproduction (Chailakhyan, 1968).

Chailakhyan (1968) reviewed that there are four groups of hormones and physiologically active compounds presently known in plants: (a) auxins, (b) Kinins and nucleic acid metabolites, (c) gibberellins and (d) anthesins, metabolites which are necessary for flower formation.

Flower initiation which is the cumulative effect of successive light and dark reactions, is the first major step in the reproductive growth of plants. The metabolic changes occurring in light and dark have a quantitative regulatory effect on flowering, but the reaction which results in the formation of flower inducing hormone is more important and decisive and is yet to be unravelled. Chailakhyan (1937) believed that flowering in plants is caused by a flower forming hormone the "florigen". He also considered that florigen comprises two components—the gibberllins and the anthesins (Chailakhyan, 1968). A number of gibberllins and gibberellin like substances have been extracted from a number of plants (Lang, 1970) but the anthesins, assumed to be responsible for flower initiation, have not
been extracted and isolated as yet.

In the present investigation, the lower dose of NAA and IAA was found to cause early flowering whereas the higher dose was found to have inhibitory effect on flowering in *Boerhaavia diffusa*. Cholodny (1939) also formulated the view that high auxins-levels in the soybean plant may be unfavourable to flowering. Salisbury and Ross (1986) observed promotion in flowering in some bromeliads including the pineapple, with the application of auxin. In the bromeliads, IAA was found relatively ineffective while NAA was found effective in promoting flowering. Mathur (1963) also reported that treatment of NAA caused early flowering in cotton.

Lincoln and Hamner (1958) applied gibberellic acid to cocklebur and showed improvement in flowering. Harada and Nitsch (1959) showed that Japanese variety of Chrysanthemum induced to flower by GA₃. Stowe and Yamaki (1959), in reviewing the GA effect on flowering, concluded that it acted primarily on elongation. They also suggested that GA in some cases incidentally releases flowering response, but dose not act primarily on flowering. Burk and TSO (1958) induced rosette species of tobacco to flower with GA. Similar results were also obtained in spinach and *Pyrethrum* and *Pharbitis nil* (Bennet et al., 1966) with GA.
Pharis et al., (1977) observed that most conifers require several years before they ripeness to respond, but spraying of GA₃ was able to induce the formation of male strobili on Arizona cypressus arizonica) when plants were only 55 days old, Mathur et al., (1969) also obtained similar results in flowering of Dahlia with GA₃. Mathur and Mittal (1964) obtained greater flower formation in cotton with the application of GA₃. Xhu and Davis (1977) working on pea lines (dwarf and tall) found an increase in flowering with GA.

**Fruit and Seed Formation:**

In the present findings, the treatment of different concentrations of Naphthalene acetic acid, Indole acetic acid, Gibberellic acid and NPK mixture found to increase fruiting, fruit setting and finally the number of fruits and seeds in Punarnava (Boerhaavia diffusa Linn.). Similar results were also reported by Bajpai and Thakur (1957). They reported that application of NAA and IAA was found to increase fruit setting in loquat. Eaton (1950), Dastur and Prakash (1954) and Bhatt and Date (1955) have also obtained significant increase in boll-setting in cotton with treatments of Naphthalene acetic acid.

Malkani and Asana (1958) and Mathur (1963) have reported significant increase in boll-setting in cotton as a results of treatment
of auxins. Randhawa et al., (1961) also observed better fruit-setting by reducing fruit drop with Naphthalene acetic acid in sweet orange (*Citrus sinensis* Linn.). Sen and Verma (1962) recorded longer and heavier fruits in mustard T$_{102}$ with Naphthalene acetic acid. Crane (1964) also reviewed that fruit-setting by growth regulators is a function of their auxin activity. The significance of auxin in fruit-setting has been recognised for over past 30 years. Fruit such as egg plant, fig, tomato, cucumber, and pepper may be set by auxin application and their ultimate size and development may not be greatly different from their pollinated counterparts. Other fruits, however, such as the apple, apricot, avocado and pear may be set by auxin application.

Walhood (1957) and Walhood and Hoover (1958) have emphasized the role of gibberellin on boll-retention in cotton and found that application of gibberellin to flowers at anthesis or to one or two days old bolls negated or reduced flower abscission and boll-shedding and promoted boll-retention. Krishnamurthy *et al.*, (1959) obtained increase in fruit-setting, size and quality of Pusa seedless variety of grape with Gibberellic acid. Singh and Randhawa (1959) working on strawberry (Pusa early variety) obtained an increase in length of peduncle, fruit formation and total yield with Gibberellic acid. Similar results were also
obtained in Phalsa (Grewia asiatica Linn.) with the application of GA (Randhawa and Khanna et al., 1959). The increased fruit-set, reduced fruit-fall and increased number of fruits/plant were also recorded in sweet lime (Citrus limettiodes, Tanaka) with GA (Randhawa and Dhuria, 1959). and in sweet orange (Citrus sinensis, Osbeek) var. Jaffa, pine apple and mosambi (Randhawa et al., 1961). Randhawa and Sharma (1962) obtained highest fruit-set with Gibberellin in sweet orange varieties Jaffa, pineapple and mosambi. Sen and Verma (1962) working on mustard variety T102, observed an increase in flowering, fruit size and yield with the application of Gibberellin. Van Overbeek (1962) obtained increased fruit set grape with Gibberellin. Crane (1964) also obtained better fruit set in tomato, fig, cucumber, egg plant, pepper, pear, apple and currant with the application of Gibberellin than auxins. The grape, particularly Black corinth variety, has been widely and erroneously cited as responding to Gibberellin application by increases in fruit-set.

Similar to plant hormones, NPK mixture was also found to increase the fruits and seeds per plant applied as basal dressing to the Boerhaavia diffusa Linn. Singh and Pandey (1970) also obtained satisfactory yield of chillies with NPK application. Similar results were also obtained in Capsicum with NPK application by Bangash
and Shaikh (1972).

Shekhawat et al., (1975) also reported that dwarf wheat varieties produced significantly more grain yield when supplied with NPK fertilizers. Tewari et al., (1980) also observed an increase in grain number and grain yield in dwarf wheat cv. HD 1982 with the application of NPK fertilizers 13:13:20; 15:15:15 and 20:10:10 respectively. Similar results were also obtained in Linseed (Bursukov and Lenenko, 1982) and in mustard (Swamy and Bajaj, 1988) with NPK application.

The interaction effect of NPK + NAA; NPK + IAA and NPK + GA$_3$ was found superior in respect of growth, flowering, fruit setting and seed formation in comparison to hormones (NAA, IAA, GA$_3$) and NPK mixture applied alone.

**Crude Extract of Root:**

The crude extract of root of *Boerhaavia diffusa* Linn. having the alkaloid Punarnavine responsible for the control of urinary disorders was found to increase with the increase of root growth with the application of NPK mixture, NAA, IAA, GA$_3$ and their interaction. The interaction of NPK$_{80}$ + NAA$_{40}$ was found most superior to all other treatments of NAA, IAA, GA$_3$ and NPK applied alone in respect of more root growth resulting an increase in root extract.
As regards the medicinal value of the *Boerhaavia diffusa* Linn., it has the extra importance in controlling different types of diseases. Each part of this plant has its specific medicinal value. The leaves of the Punarnava (*Boerhaavia diffusa* Linn.) belonging to the family Nyctaginaceae have a sharp taste, appetiser, alexiteric, used in Ophthalmia for eye wounds, and in the pain of joints. The seeds are tonic, expectorant, carminative, useful in muscular pain, lumbago, scabies, scorpion-string, purify the blood and hasten delivery. It is a popular indigenous drug commonly used as a diuretic and urinary analgesic in the treatment of Oedema (Kritikar and Basu, 1933; Nadkarni, 1954).

Kritikar and Basu (1944) also reported that punarnava has been described to be bitter, cooling, astrigent to the bowels, useful in billiousness, blood impurities, leucorrhoea, anaemia and inflammation. According to Chopra et al., (1956), the root of *Boerhaavia diffusa* Linn. has an extra ordinary medicinal value such as diuretic, laxative, expectorant, in asthma, stomachic, in Oedema, anemia, jaundice, ascites, anasarca, scanty urine, internal inflamantidote to snake-venom. Singh (1969) also described it as anti-inflammatory, antibacterial drug specially against regenerative effect of kidney.

According to Haines (1963), the root of *Boerhaavia diffusa*
Linn. is used as laxative, diuretic and stomachic. It is also used in disease of heart and kidney as well as in gonorrhoea and dropsy (Ramaswamy and Razi, 1973). Bhalla et al., (1971) revealed that the acetone extract of the \textit{Boerhaavia diffusa} was most potent both on carrageenin-induced Oedema and formaldehyde-induced arthritis.

Mudgal (1974) found diuretic and anti-inflammatory activity in root and leaves. Mudgal et al., (1977) also found that increased rate of biosynthesis of active principle (alkaloid) is responsible for the increased medicinal properties of these plants.

Therefore, keeping in view the extra ordinary medicinal value of leaf, stem, root and seeds of \textit{Boerhaavia diffusa} Linn. specially the property of controlling urinary disorders, it is necessary to increase the vegetation of this plant to fulfil the demand of the people for medical use. Our experiment is helpful in increasing the vegetation of this plant by cultivating the plant with the help of NPK fertilizer (15:15:15), Naphthalene acetic acid, Indole acetic acid and Gibberellic acid and their interaction to meet the medical demand of the people of the country.

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