1. INTRODUCTION

INTRODUCTION

1.1 GENERAL INTRODUCTION :

It is a well known fact that whole of the world is facing the serious problem of population. World problem is increasing with horrible rate. This already blocks the betterment of millions of poor people, hampers progress of many poor countries and threatens affluence of the rich. If the rate of the population is allowed to continue like this, one day there will be a problem of survival of human species itself. This situation lead to the serious thinking of controlling populations all over the world.

To check this population explosion, the only effective way is to bring down birth rate. So far maximum efforts were made on females while the males were comparatively, ignored. But now attention is being given to male reproductive system. Many synthetic preparations are being tried to control male fertiltly , but many of which are not suitable for humans, since they produce number of side effects in the animals. (For Review see Mann; 1964, Gomes, 1970; Patanelli, 1975).

In contrast to synthetic preparations, the plant preparations, tried so far produce practically no side effects. Hence in recent years reproductive physiologists all over the globe are taking interest in plant preparations to control human fertility.

1.2 REVIEW OF MEDICINAL PLANTS HAVING ANTFERTILITY EFFECTS :

There are number of plants whose extracts were shown to be affecting the fertility. The following is brief review of the work on the effects of some plant extracts on fertility in males.

Lithospermum (Plunkett Noble, 1951), Punica granatum (Gujaral et al; , 1960) , Butea monosperma (Dreischbach, 1963, Khanna & Chaudhary 1968, Awati 1985), Hippophae salicifolia (Joshi et al; 1965), Ananas comosus (Bhaduri et al; 1968), Toro, 1984, Chinoy et al; 1988), Butea frondosa (Razdan et al; 1969, Srivastava, 1982), ROC-101, a herbal preparation composed of three different plants (Munshi & Rao, 1971), Oscimum sanctum (Kasinathan et al; 1972, Seth et al; 1981, Khanna et al; 1986), Channabis, (Dixit et al; 1974), Opium seeds (Vyas and Singh, 1976), Malvaviscus conzanttii, (Dixit, 1977, Bhargava, 1988), Aristolochia indica (Pakrashi and Pakrashi, 1977), Momordica charantia (Biswas et al;, 1977), Blighia sapida (Saxena et al;, 1977), Hibiscus rosasinensis (Kholkute, 1977, Singwi and Lall, 1980), Vitex negundo (Singh et al;, 1978, Sohoni, 1985), Vinca rosea and Embelia ribes (Chauhan et al;, 1979), Calatropis procera (Gerg, 1979), Artobotrys odoratissimus (Prakash, 1979), Papaya seed (Das. 1980; Chinoy and Sondarva, 1988), Allium sativum (Dixit and Joshi, 1982),

Plumbago zeylenica (Bhargava, 1984, Jadhav, 1988), Daucus carota (Shah, 1985), Gossypol (Nair et al;, 1988, Bhiwgade and Nair, 1989; Nair and Bhiwgade, 1989), Piper betle (Hiremath, 1988, Adhikary et al;, 1989), Eugenica jambolana (Rajasekaran et al;, 1988), Andrographis paniculata (Akbarsha et al;, 1988a; 1988b; 1988c; 1990), Solanum xanthocarpum (Rao, 1988), Celastrus paniculatus (Wangoo, 1988), Syzygium cumuni (Ambaldage, 1990) reported to have antifertility effects in various animals.

1.3 REASONS THAT LEAD TO THE UNDERTAKING OF THE PRESENT WORK :

A critical evaluation of available literature on the plant extract induced aspermatogenesis shows that :-

i) Intention of most of the studies is the "distruction" caused in the seminiferous tubules by the active principle in the plant extract. But very little is known about the mechanism of such "distruction".

ii) Histological details of plant extract induced aspermatogenesis are made available, but comparatively less information is available on the metabolic changes in the testis and accessory organs. Though changes in enzymes, proteins etc. are studied in many cases, all these parameters have not been studied in case of single plant, having antifertility effects.

It is hence felt desirable, to study all the reproductive organs simultaneously in a study to find out how active principle from plant extract induce damage in them.

It is with this view, it was decided to study the effects of Picrorhiza kurroa extract on male reproductive organs of albino rats. To make the study consolidated and as complete as possible, it was decided to study histological changes, alteration in lysosomal enzyme, non-lysosomal enzyme, in testis and the accessory reproductive organs of albino rat.

1.4 CHOISE OF THE PLANT :

For the present investigation Picrorhiza kurroa plant was selected .

1.4.1 BOTANICAL CONSIDERATION :

Picrorhiza kurroa

Division	- Spermatophyta
Subdivision	- Angiospermae
Class	- Dicotyledonae
Subclass	- Gamopetalae
Series	- Bicarpellatae
Order	- Personales
Family	- Scrophulariaceae
Genus	- Picrorhiza
Species	- kurroa

Plate No. 1

KUTKI

Picrorhiza kurroa

VEGETATIVE CHARCTERS OF FAMILY :

A low more or less hairy herb with perennial woody bitter stock.

Leaves Subradical, spathulate, serrate.

Flowers Spicate on radical leafy flowering stems, bracteate, ebracteate, white or blueish,diamorphic (a longer and shorter stamened form)

Sepals 5, lanceolate, imbricate in bud.

Corolla of the long stammened form, short, membranous, subequally, 5-deft to the middle lobesovate, acuminate, ciliate, of the shorter, Stamened. corolla-tube curved, broad, limb 2 lipped, upper lip longer subgaleate emarginate, lower of 3 shorter ovate acute lobes the middle on/smallest.

Stamens 4, filaments in the longer stamened, very slender,four times as long as the corolla, of the shorter stamened stout, 2 upper shorter than the upper lip of the corolla, with the anthers under the hood, 2 lower exserted, anther-cells sub divergent confluent at the tip.

Ovary 2 celled, many-ovuled style of the long stamened long & slender, stigma simple, of the shorter stamened stouter and shorter, stigma capitate exserted.

Capsule Ovoid, turgid, acute, septicidal and loculicidal, margins of the valves inflexed, exposing the columnar placentiferous axis.

Seeds of an oblong curved nucleus, enclosed in the large bladdery loose hyaline reticulated testa.

P-Kurroa Benth, Scroph, Ind. 47 and in DC. prodr X 454, Royle I.U. 291, t.71. Valeriana Lindleyana, Wall. Cat 404 (corrected to Veronica P 23).

Alpine From Kashmir to Sikkim, altitude - 15000 ft. common.

Root Root stock as thick as the little finger 6-10 inch long. clothed with withered leaf-bases.

Leaves 2-4 inch rather coriaceous, tip rounded, base narrowed into a winged sheathing petiole.

Flower Flowering stems or scapes ascending, stout, longer than the leaves, naked or with few bracts below the inflorescence.

Spikes 2-4 inch long, subcylindric, obtuse many flowered, subhirsute, bracts oblong or lanceolate, as long as the calyx.

Sepals 1/4 inch long eiliate.

Corolla of short stamened form 1/4 - 1/3 inch long with longer filaments 1/3 inch long of the longer

stamened from 1/4 inch with filaments 3/4 inch long.

Capsule 1/2 inch long.

1.4.2 BIOLOGICAL PROPERTIES :

It is considered to be a valuable bitter tonic, almost as gentian. It is antiperiodic, cholagogue, stomachic, laxative in small doses and cathartic in large doses. It is reputed to have benefical action in dorpsy. Alkoholic extract of the roots are active against Micrococcus pyogens var. aureusand Escheria coli (Datta and Mukherjee, 1050; Kirtikar and Basu, 1980). It increases weight of liver significantly (Kumar & Tripathi, 1987). Administration of Picrorhiza kurroa simultaneously with p-berghei infection showed significant protection against hepatic damage in Mastomys natalensis (Ramesh chander et al; 1990).

1.4.3 CHEMICAL COMPOSITION :

It contained a glucosidal bitter principle - kutkin (B-1-Vanilloyl-6-Cinnamyl-d-glucose, C_{23}, H_{24}, O_{11}; $2H_2O$). M.P. 211^{o}C. A non-bitter substance Kurrin (D-mannitol), vanillic acid, an alcohol-Kutkiol ($C_{40}H_{82}O$) M.P.118 ^{0}C and a sterol - Kutki sterol $C_{12}H_{40}O$) M.P. 124^{o}C. (Chopra and Ghosh, 1934; Rastogi et al;, 1949; Rastogi and Dhar, 1959).

1.5 REASONS FOR SELECTING PICRORHIZA kURROA FOR PRESENT INVESTIGATION :

Picrorhiza kurroa is tried in females and found to be useful in problems of mammary glands (Sharma, 1986) but the plant is not at all tried on male reproductive system. Renowened Ayurved practitioners from Solapur, after personal discussion with them, also seem to be hopeful about the possible infertility potency of Picrorhiza kurroa.

There are abundant reports which showed that steriods of synthetic origin are known to alter the structure and functions of male reproductive system.

Picrorhiza contains a phytosteroid Kutki-sterol. Phytosteroids are known to affect reproductive system of males (Pakrashi and Pakrashi, 1977; Chouhan et al;, 1989, Bhargava, 1984; Toro, 1984; Jadhav, 1988; Reddy et al; 1989; Sinha and Mathur, 1990).

Hence it was thought desirable to study effects of Picrorhiza kurroa extract in the present investigation.

1.6 CHOICE OF THE PARAMETERS OF STUDY :

It was decided, as it was mentioned earlier, to study Picrorhiza kurroa extract induced aspermatogenesis in adult albino rats employing well known and recent histological and biochemical techniques. The following parameters were chosen for these studies:—

1. Histology

2. A lysosomal enzyme (Acid phosphatase)

3. A non-lysosomal enzyme (Alkaline phosphatase).

The reasons in brief why the above parameters have been selected for the present study are;-

Histology -

The funcitional significance of any organ or system, normal or affected, will be known only when the structural alterations are studied and this is possible only with histological studies. Picrorhiza kurroa extract induced histological alterations in the testis and accessory organs will reveal the exact locus of action during such induced aspermatogenesis.

Lysosomal enzymes (Acid phosphatase) :

Lysosomes and their acidic hydrolysing enzymes have definite role in normal testicular functioning in various vertebrates (Fishman and Baker, 1956, 1968; Hayashi, et al;,1958a, 1964, Novikoff et al; 1963, Tice and Barnett, 1963, Dalcq and Bertrand, 1968, Elkington and Blackshaw, 1973, 1974, Gomes and Van Demark, 1974, Majumdar et al;, 1975; Chang et al;, 1975, Baile, 1975, Ericsson, 1976). The conclusion drawn by the observations of these workers, in brief, can be summerised as -

1. Various lysosomal and hydrolysing enzymes are localised in different testicular cellular components.

2. The lysosomes and their acid hydrolysing enzymes exhibit interesting changes during various functional states of the testis.

3. There is an inverse relationship occur between spermatogenesis and acid hydrolysing enzymes (Dott, 1973). In the review of the work on the lysosomes in the seminiferous tubules, he stated that " In the testes the lysosomal activity is involved in secretion, cell division and in the removal of unwanted material, but the particular group of enzymes involved is probably different".

4. In variety of vertebrates, the lysosomes and various acid hydrolysing enzymes have been reported in Leydig cells. The enzymes have been related to the functional state of the Leydig cells.

5. Considerable amounts of acid hydrolysing enzymes are present in the Sertoli cells. These cells are involved in the heterophagic digestion of germ cells, globules of residual spermatid cytoplasm left behind in the release of spermatozoa.

6. Vast literature is cited, on close involvement of lysosomes and their acid hydrolysing enzymes in the lytic processes by Dingle and Fell (1969, 1971).

7. Considerable activities of lysosomal acids hydrolases

have been reported in the accessory glands of the rat. Lysosomal enzyme activities reflect the functional states of these organs as well as they respond to the androgen level; if andorgen levels decrease the enzyme contents also decrease and vice-versa. Thus behaviour of lysosomal enzyme reflects on the hormonal contents of the male (Hopkins, 1970).

Another reason for selection of a lysosomal enzyme for the present work was that during plant extract induced aspermatogenesis some lytic changes do occur in the testis. Hence it will be very interesting to study the lysosomal enzymes in such process.

Non-Lysosomal Enzyme (Alkaline Phosphatase):

Alkaline phosphatase is a non-lysosomal enzyme, hence it is expected to give a good contrast to the behaviour of the lysosomal enzymes during plant extract induced aspermatogenesis.

1.7 PRESENTATION OF THE THESIS :

It was selected to divide the present thesis into five chapters. The first chapter being on introduction giving a review of the literature on agents causing aspermatogenesis in the vertebrates, reasons that lead to undertaking of the present work, details of the plant, Picrorhiza kurroa, its properties and reasons for the

selection of various parameters for the present, investigation. The second chapater contains detailed description of the materials and methodology and techniques employed in the present investigation. Third chapter deals with the alterations caused by Picrorhiza kurroa in the histoarchitecture of testis and accessory repreductive organs. Fourth chapter deals with biochemical alterations in testes and accessory reproductive organs with respect to alterations in acid phosphatase and alkline phosphatase caused by administration of Picrorhiza kurroa extract, for a period of 48 days.

Third and fourth chapters contain a brief review of earlier work, detailed report of observations and discussion; the observations have been duly compared with the available literature on effects of various antifertility agents and certain conclusions have been arrived at. The observations are well supported by tabular data, graphical representation and by photomicrographs of histological observations. The fifth chapter of the thesis contains summary with concluding remarks. Bibliography of exhaustive literature cited in the thesis, is at the end.