CHAPTER – III

EXPERIMENTAL LEUKAEMOGENESIS IN MICE: Effects of administration of Ethyl Nitrosourea in young mice.
Experimental Leukemogenesis in Mice:

Leukaemia and lymphomas of laboratory mice, rats and other lower animals have been found useful models for investigations into aetiology, genetics, mechanisms of target cell transformation, cell renewal kinetics, experimental therapy and many other problems relating to leukaemia as a disease of the haemapoietic system. The principles and knowledge gained from such investigations have been implemented in studies of leukaemia in man, and have served as a basis for the design of experimental treatment and the development of anti-neoplastic drugs.

Induction of Experimental Leukaemia:

Studies of spontaneous leukaemia in various species of animals have paralleled clinical studies of leukaemia in man to date and back to the last century [Forkner 1938 (247), Engelbert 1942 (248)]. Numerous attempts at transmission of human leukaemias to animals met with failure. Transmission of animal leukaemia and lymphoma by virus first succeeded in birds at the beginning of this century [Ellerman and Bang 1908 (249), Rous 1910 (250)]. Krebs et al (1930) (251) were motivated by the relatively high incidence of leukaemia among radiologists and started research on the induction of leukaemia in laboratory animals by ionizing radiation which began in the 1930s. By that time Morton & Mider (1938) (252) established induction of leukaemias and lymphomas in experimental animals by carcinogenic chemicals and before the war the existence of mammalian tumour viruses was demonstrated (Rich, 1968) (253). The ground-work for investigations into mammalian leukaemia was laid with the development of inbred strains of mice (Little 1941) (254). This permitted studies of genetic influences on leukaemia
induction, selection of high and low leukaemia strains and the use of transplanted leukaemias as convenient and reproducible model systems.

**Effects of Administration of Ethyl Nitrosourea in Young Mice:**

Of the newly synthesized oncogenic N-N'-Nitroso-compounds, ethyl nitrosourea (ENU) has been shown to be a potent resorptive carcinogen. Incidence, location and types of neoplasms produced with ENU in rats depend greatly upon age, dosage, strain and to a certain extent upon the physiologic state of the animal e.g. pregnancy. Druckrey et al (1966) (255) produced tumours of various organs (of the hematopoietic system, 4 of the brain and 2 each of the spinal cord, small intestine, uterus and mammary gland) in adult BDIX rats with weekly intravenous injections of 10mg/kg ENU upto a total dose of 250mg./kg. Inoculating pregnant female rats with single intravenous doses of 20-80 mg./kg. ENU resulted in a predominance of neoplasms of the genital tract (ovaries, uterus and vagina), indicating a higher susceptibility of the genital tract to the carcinogenic effect of ENU during pregnancy. The influence of age upon the incidence, location and tumour types was clearly demonstrated by Druckrey et al (1966) (255) in an experiment in which BDIX rats, in age groups of 1, 10 and 30 days, were inoculated with a single dose of 5-80 mg./kg. of ENU. The significant findings of that experiment were the demonstration of decreased susceptibility of the older age groups for the oncogenic effect of ENU and the high incidence of tumours of the central and peripheral nervous system in the 1 day old rats. This trend of decreasing susceptibility to the oncogenic effect of ENU became even more pronounced in adult rats. Doses of 20-80 mg./kg. ENU resulted in none or a few tumours of the central nervous system (CNS) reaching an incidence of approximately 20% at dose levels of 70 and 80 mg./kg. and with very high single doses of 140 mg./kg.
neurogenic tumours were produced in 45% of the animals and with 200 mg./kg. in 60% of the animals.

Ivankovic and Druckrey using BDIX rats, demonstrated that transplacental production of tumours was not possible before the twelfth day of gestation and their initial work indicated an increased susceptibility for induction of neurogenic tumours with advancing pregnancy, culminating toward the termination of the gestation period. Induction of leukaemia, however, was pursued with less importance. The present chapter is devoted to study the ENU effects towards leukaemia induction.

**Materials:** For the establishment of experimental leukaemia induction, following materials are required:

1. Swiss Albino inbred Mice (supplied from the School of Tropical Medicine) ageing 5-10 days.
2. 1 ml. Syringe.
3. Ethyl Alcohol (Bengal Chemical).
5. N-N' Ethyl Nitrosourea (ENU), ascorbic acid.

**Methods: Preparation of ENU:**

For the induction of leukaemia in mice N-N ethylnitrosourea (ENU) was inoculated at the dose rate of 50 mg./kg. body weight at the first week and solutions were prepared in saline and pH adjusted to 4.8 to 5.6 with ascorbic acid.
LEGENDS TO FIGURE:

Fig. 1:

The survival data of different groups of mice including ENU induced leukaemia and those receiving single or multiple doses of Biological Response Modifiers (BRMs) in combinations. Significant survival protection has been noted following BRMs therapy of leukaemic mice (Chapter - III).
Protocol:

(1) At the last trimester of pregnancy, ENU was inoculated into pregnant mother intraperitoneally.

(2) In some animals within the 10th day of birth 2nd. ENU was challenged with a dose of 30mg./kg. B.W. intraperitonealy.

(3) After 6-8 months of ENU challenge experimental leukaemia was induced into the mice as revealed from normal leishman stain of the smear. Plate (1a,b,c).

Results: Induction of leukaemia in mice under the present event was of a mixed type, although, majorly lymphoblastoid in nature (Plate 1a,b,c). However, presence of myeloblasts were also noticed. Apart from leukaemia induction in rat, mice were also shown to be suitable for leukaemia induction. The percentage induction in leukaemia was more than 60%. (Fig. 1)

External Manifestation and Behavioural Changes: After induction of leukaemia by N-N ethylnitrosourea around 15% of the animals died due to acute toxicity 60% of the total animals developed leukaemia and showed,

(i) loss of hair,
(ii) infection of the foot and mouth and the eyes,
(iii) bleeding manifestation,
(iv) shorter life span,
(v) decreased intake of food and water,
(vi) mutilated limbs with infrequent movements,
(vii) Dehydration, Diarrhea, anorexia,
(viii) Infections on the different-parts of the body.
LEGENDS TO FIGURE:

HISTOLOGICAL FEATURE:

Plate 1: The peripheral blood smear of mice with ENU induced leukaemia (6 to 8 months following ENU treatment): (a) shows presence of predominating lymphoblasts (b) occasional myeloid blasts were also seen.
(b) **Infective death**:- It was evident that nitrosourea-compound is a potent immuno-suppressive agent. Due to the carcinogenic effect of ethylnitrosourea neutropenic condition is produced. The causes of death in such animals were found to be 25% due to the secondary infections rather than the disease itself. In many cases the spread of malignancy was observed in tissue specific areas including lung, liver, spleen, intestine even in eyes.

(c) **Survival Study**:- Development of experimental leukaemia into the animals by the application of carcinogenic ethylnitrosourea (ENU) showed decreased survival rate of animals. Affected animals died within 150 days of the total lifespan of 450 days. After the ENU challenge 60% of the animals developed mixed type of leukaemia within 6-8 months. Due to appearance of the malignant blast cells animals suffered from an immunocompromised state with secondary infections. Leukaemic effect with secondary infections also resulted in decreased survival of animals.

(d) **Histological Feature**:- Blood film prepared from the experimental and normal animals showed different picture after leishmann staining. ENU induced experimental animals provided mainly blastoid cells in the blood film after 6 months of ENU challenge. Histological studies with stained film indicated predominating lymphoblastic cells in the blood film. From the blood picture it has further been established that a mixed type of blast cells with predominance of lymphoblastic cells appear in these experimentally induced leukemic mice.

**Comments**: Attempts to established an experimental leukaemic model of mice helped to investigate the conditions of disease with special reference to leukomogenesis and secondary infections. Carcinogenic as well as teratogenic nature of ethylnitrosourea showed a toxic and
immunosuppressive susceptibility of secondary infections. This caused enhanced death rate, of animals that indicated needs for management of infections are in leukæmic conditions. The study maintained in this chapter infact, helped elucidating standard method for leukemia induction in experimental animals. Such method was somewhat modified from that if others working ENU in pregnant animals. Present attempts also shows that ENU administration in young animals can induce leukemia.