CHAPTER VI

CERTAIN ELEMENT PROFILE IN WHITE MICE BEARING DALTON'S LYMPHOMA WITH OR WITHOUT BRH_2

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Elements play an essential role in a number of biological processes through their action as activator(s) or inhibitor(s) of enzymatic reaction by competing themselves for protein binding site, by influencing the permeability of cell membranes or through other mechanisms. It is therefore, reasonable to assume that these minerals exert action either directly or indirectly on the carcinogenic process. Metals ions can interact with nucleic acids to influence base pairing errors or frame shift mutations, by deletion leading to cellular transformation, for example, Mg, Mn, and Zn are cofactors of many enzymes especially RNA & DNA polymerase.

Many of the trace metals are associated with enzymes involved in vital physiological processes. Variation in the quantity of trace elements like Fe, Zn, Mn, and Cu was reported in different forms of malignancy (Ranade and associates, 1984). It bears significant importance as marker in cancer. Schwartz (1975) attempted to show that higher levels of various elements in malignant tissues. The presence of specific elements in human subjects is an indicator of cancer (Zhang et al., 2001).

Trace metals which occurs as micro constituents of cells and tissues may either advance or retard the kinetics of anabolic and catabolic enzymes. The recognition that variations in the trace metal content of cells or tissues can produce pathological state has been gathering momentum with a renewed understanding of the role of elements in the physiology. Trace elements Selenium (Se), Zinc (Zn) and Copper (Cu) play an important role in structural and functional cofactors of various enzymes crucial for the various biochemical cell activities and the integrity of various apparatus (Guillard et al., 1980; Marklund et al., 1982; Halliwell et al., 1984).

Variation of Cu concentration in different organs (Bloomer & Lee, 1978; Owen, 1964) and with age was investigated (Evans et al., 1970, 1974; Everett et al., 1964). It was established that in cancer patients' serum Cu concentrations are of considerable importance in assessing the activity and prognosis of the diseases (Hrgovicic et al., 1973; Roguljic et al., 1980; Drodz et al., 1986; Zhi Jun, 1988). Elevated serum copper and decreased serum zinc concentrations have been detected in patients with sarcoma (Breiter et al., 1978) and carcinoma of the digestive system (Inutsuka and Araki, 1978). Enhanced Cu concentration in ovarian carcinoma has already been described.

Variation of Cu concentration in human breast tissue was reported (Ranade et al., 1989; Rizk and Sky-Peck, 1984). It has been observed that higher levels of Cu and Ni and significantly lower levels of Zn and Mn in malignant

tissues (Wan – Shun 1992). The association of Cu deficiency and impaired glucose metabolism with inflation has already been demonstrated (Abdel Mageed, 1990b).

Zinc and copper are two important trace elements of the tissue. Zinc is necessary for growth, appetite, testicular maturation, skin integrity, mental activity, wound healing, prevention of lipid peroxidation and immunocompetence (Burch et al., 1978). Zinc is involved in the activity of at least 90 enzymes. The stability of various biological macromolecules is dependent on or increased in presence of Zinc (Wong et al., 1972). Andronikaskvili et al. (1972) concluded that zinc is rather important in some forms of neoplastic growth. It has been established that zinc alone is responsible for nucleic acid biosynthesis in regenerating liver (Fujioka and Liberman, 1964 and Weser et al., 1969) while Rubin (1972) defined a correlation of Zinc content with Rous sarcoma development.

Lal et al.(1989) observed decreased amount of Cu and Zn in cancerous esophagus while Diez et al. (1989) suggested increased amount of Cu and Cu / Zn ratio in malignant lung tissues. Elevated Zn in cerebral tumors (Schicha et al., 1972), primary brain tumors (Muller et al., 1988) and breast tumors (Rizk and Sky – Peck, 1984) have also demonstrated low level of plasma Zinc in tumor bearing rats (Mills et al., 1986). Zinc deficiency in nitrosamine induced oesophageal carcinogenesis has been reported by Frong et al. (1988). High incidence of oesophageal cancer in Zn – deficient

human being has already been suggested (Kmet and Mahboubi, 1972). Generally, blood Zn level was found to be decreased in neoplasia (addink and Frank, 1959) and especially in bronchogenic carcinoma (Davis et al., 1968). The lowest level of serum Zn was recorded in prostatic and some other form of cancers (Feustal et al., 1989a – 1989b; Zhen-Geng, 1991). Earlier Koch et al. (1957) reported elevated values of Zn in Hodgkin's disease, chronic lymphocytic leukemia, lymphosarcoma and other related conditions. However, Addink & Frank (1955), reported decrease in the level of blood Zn in the tumors and similar decrease was also observed by Issel et al. (1981).

More recently evidence has been presented to support the contention that Selenium may have anticarcinogenic properties in rats and mice. Selenium is increased in patients with leukemia or cancers of the reticuloendothelial system. In particular, Se is a constituent of the glutathione peroxidase, and Zn and Cu are cofactors of superoxide desmutase. These enzymes act as scavengers against free radicals of oxygen that form in various modes in the body and protect from different cellular damaging events. Indeed derangement of the cell membrane and of DNA, exchange of chromatids, and genetic mutations can be caused by oxidative stress, and they are correlated with carcinogenesis (Guillard et al., 1980; Marklund et al., 1982; Halliwell et al., 1984). The early work of Shamberger (1970) also showed that sodium selenite inhibited carcinogenesis. Many other studies have attested to a role for selenium in protecting against carcinogenesis in animal

models. Selenium deficiency has been associated with many illness including muscular dystrophy, liver necrosis, Keshan diseases, and other conditions that may be prevented through selenium supplementation (Burk, 1983). Selenium has been found in many epidemiological studies and in other experiments to play an important preventive role in carcinogenetic processes (Schrauzer, 1976).

A case control study found that prostate cancer patients had lower plasma selenium than that in matched controls, but with insignificant difference (West et al., 1991). A nested case control study of a large cohort found that higher toenail selenium levels were associated with a reduced risk of advanced prostate cancer (Yoshizawa et al., 1998). Another study found no association between toenail selenium and breast cancer or prostate cancer though they observed a statistically significant inverse association between toenail selenium level and the risk of colon cancer for both genders combined and for female subjects (Ghadirian et al.,, 2000). One ecological study suggested a positive association between serum selenium and breast cancer risk (Guo et al., 1994). Another correlation study showed that the serum selenium of colon cancer cases was significantly lower than that in the general population (Caroli et al., 1994). Animal and laboratory studies indicate the protective role of selenium in the etiology of malignancies, but this role in epidemiological studies exhibits contradictory results, may be due to methodological problems (Ghadirian et al., 2000).

The relative cancer risk in the lowest quintile of serum selenium (0.107 µg/ml) was twice that of subjects in the highest quintile (0.172 µg/ml). The risk associated with low selenium was even greater if serum vitamin E and retinol were also low. Studies indicate that plasma selenium levels below 0.10 ppm are associated with increased cancer risk, while significantly lower cancer risks are observed for plasma selenium levels of 0.2 ppm or more.

A number of epidemiological studies have now been reported which show no relationship between selenium status and cancer risk (Levander, 1987). Moreover, a recent analysis of the relationship between selenium and cancer suggests that "the question of whether selenium protects against cancer is still wide open" (Willett et al., 1983).

However, the relationship between the element level and the metal-based drugs like Cu in Dalton's ascites lymphoma is not much known. Therefore, an attempt has been made to evaluate the quantity of certain elements in Dalton's lymphoma bearing C_3H / He mice with or without BRH_2.

Materials and methods:

The details of the methodology have been described in the chapter II. Briefly, three sets with five groups of rats were randomly selected.

CONTROL SET - I

A: Normal mice, with 0.75% aqueous 0.2 ml Carboxy-methyl Cellulose (i/p).

MALIGNANT SET - II

B: & C: Received 0.2 ml (1 x 10^7 Cell) Ascite Dalton's lymphoma (i/p), sacrificed on 10^{th} & 20^{th} day respectively.

CHEMOTHERAPY GIVEN SET - III

D: BRH2 in 0.2 ml of Carboxy-methyl Cellulose (100 mg / Kg bw) injected i/p on 1^{st}, 5^{th} and 9^{th} day after Dalton's lymphoma implanted, sacrificed on 20^{th} day.

E: Treated same as group D and sacrificed on 35^{th} day.

Results:

The distribution pattern of Copper (Cu) in different organs of both control and treated sets has been illustrated in ***table 6.1***. The Cu concentration in the liver, spleen & kidney of DAL bearing C_3H / He mice was depleted compared to their respective normal control. The depletion was highest on 20^{th} day (C) being 59.07% in liver, 51.4% in spleen and 33.7% in kidney.

But in case of bone marrow Cu concentration was increased significantly i.e. 24.03% on 10^{th} day.

Lymphoma implanted BRH_2 treated groups displayed significant alteration of Cu concentration ($p<0.01$). The liver of the DAL bearing mice on 35^{th} day exhibited 39.3% depletion of Cu concentration from the control. In contrast, the 20^{th} day and 35^{th} day spleen tissue of the same treatment series showed a significant ($p< 0.01$) rise of 98.4% and 31.3% of Cu concentration over the control. The 35^{th} day kidney of the same treatment presented highest elevation of 165.7% of Cu concentration over the normal control. Bone marrow on 20^{th} & 35^{th} day of the same treatment also showed a significant ($p < 0.01$) enhancement of 55.6% and 62.2% of Cu concentration over the normal control ***(Table. 6.1)***.

Zinc (Zn) level in different organs of mice under different treatment protocol has been presented in the ***table 6.2***. The Zn concentrations in all the tissues, e.g. liver, spleen, kidney & bone marrow on 10^{th} & 20^{th} day of DAL implanted were found to be significantly ($p < 0.01$) elevated by 16.3% and 81.3% in liver, 80.2% and 104.8% in spleen, 14.0% and 43.8% in kidney and 21.4% and 45.4% in bone marrow respectively over the control.

Among the lymphoma implanted BRH_2 treated liver of mice on 35^{th} day ($p < 0.01$) exhibited 30.5% decrease of Zn concentration compared to control. Though significantly higher Zn level was noted in the lymphoma implanted

TABLE: 6.1: Copper concentrations (μg/g wet wt.) in different organs of control and various treated groups of C_3H/He mice. Each value represents Mean ± SE of 6 animals. Figures in parentheses represent range of variation.

ORGANS	ANIMAL GROUPS				
	A Normal Control	B DAL Implanted on 10th Day	C DAL Implanted on 20th Day	D DAL + BRH_2 On 20th Day	E DAL + BRH_2 On 35th Day
LIVER	24.97±0.28 23.8 – 26.2	a 10.27 ± 0.31 (9.2 – 11.3) ** (-A 58)	a 10.22 ± 0.28 (9.5 – 11.4) ** (- A 59)	c 25.22 ± 0.27 (24.3 – 25.9) (+ A1) (+C 146)	ac 15.17 ± 0.15 (14.8 – 15.8) ** (- A 35) (+C 48)
SPLEEN	9.97 ± 0.31 (9.2– 11.2)	a 5.05 ± 0.25 (4.3 – 5.8) ** (- A 49)	a 4.85 ± 0.18 (4.1 – 5.4) ** (- A 51)	ac 19.78 ± 0.28 (18.8 – 20.7) ** (+ A 98) (+C 307)	ac 13.07 ± 0.29 (12.3 – 14.2) **(+A 31) (+C 169)
KIDNEY	15.12±0.27 (14.2–15.9)	a 11.93 ± 0.11 (11.6 – 12.3) ** (- A 21)	a 10.02 ± 0.18 (9.5 – 10.7) **(-A 33)	c 15.2 ± 0.25 (14.5 – 15.9) (+C 51)	ac 40.17 ± 0.32 (39.2 – 41.2) **(+A 165) (+C 300)
BONE MARROW	9.82 ± 0.3 (8.8– 10.9)	a 12.18 ± 0.23 (11.5 – 12.8) ** (- A 24)	10.0 ± 0.24 (9.3 – 10.9) (+A2)	ac 15.28 ± 0.28 (14.3 – 16.2) **(+A 55) (+C 52)	ac 15.93 ± 0.26 (15.2 – 16.8) **(+A 62) (+C 59)
F is significant at ** P < 0. 01, CD is significant at P < 0.05					

a, c significantly different from the group A and C.

Figures in parentheses are % of increase over control (+A), decrease (-A), malignant group (+C), (-C).

TABLE: 6.2: Zinc concentrations (μg/g wet wt.) in different organs of control and various treated groups of C_3H/He mice. Each value represents Mean ± SE of 6 animals. Figures in parentheses represent range of variation.

ORGANS	ANIMAL GROUPS				
	A Normal Control	B DAL Implanted on 10th Day	C DAL Implanted on 20th Day	D DAL + BRH_2 On 20th Day	E DAL + BRH_2 On 35th Day
LIVER	195.47 ± 2.63 (185.5– 202.3)	a 227.4 ± 1.84 (220.5–233.8) **(+A 16)	a 354.37 ± 2.21 (345.6– 361.5) **(+A 81)	a 201.77 ± 1.48 (196.7– 206.8) **(+A 3) (-C 43)	a 135.9 ± 1.7 (130.6– 142.2) **(-A 30) (-C 61)
SPLEEN	91.8 ± 2.1 (85.5 – 98.5)	a 165.45 ± 2.51 (157.8– 174.5) **(+A 80)	a 180.03 ± 2.21 (178.5 – 195) **(+A 104)	ac 112.85 ± 2.36 (103.5– 120.6) **(+A 22) (-C 40)	ac 105.45 ± 1.78 (98.5 – 111.2) **(+A 14) (-C 44)
KIDNEY	184. ± 2.17 (178.6 – 192.8)	a 210.85 ± 2.47 (202.5– 221.0) **(+A 14)	a 265.92 ± 2.66 (256.5– 274.8) **(+A 43)	ac 220.03 ± 2.68 (209.9– 229.8) **(+A 19) (-C 17)	ac 215.53 ± 3.0 (204.5– 226.5) **(+A 16) (-C 19)
BONE MARROW	95.2 ± 1.97 (87.6 –102.3)	a 115.52 ± 2.8 (107.2– 127.0) **(+A 21)	a 135.55 ± 2.57 (128.4– 146.2) **(+A 42)	a 128.62 ± 2.74 (119.5– 139.0) **(+A 35) (-C 5)	ac 120.73 ± 2.52 (111.3– 129.5) **(+A 26) (-C 11)
F is significant at ** P < 0. 01, CD is significant at P < 0.05					

a, c significantly different from the group A and C.

Figures in parentheses are % of increase over control (+A), decrease (-A), malignant group (+C), (-C).

TABLE: 6.3: Selenium concentration (μg/g wet wt.) in different organs of control and various treated groups of C_3H/He mice. Each value represents Mean ± SE of 6 animals. Figures in parentheses represent range of variation.

ORGANS	ANIMAL GROUPS				
	A Normal Control	B DAL Implanted on 10th Day	C DAL Implanted on 20th Day	D DAL + BRH_2 On 20th Day	E DAL + BRH_2 On 35th Day
LIVER	75 ± 1.63 (70 – 81)	a 50.17 ± 2.23 (42 – 57) **(-A 33)	a 45 ± 2.54 (35 – 52) **(-A 40)	ac 155 ± 2.4 (147 – 162) **(+A 106) (+C 244)	ac 165 ± 1.53 (160 – 171) **(-A 120) (+C 266)
SPLEEN	120 ± 2.35 (110 – 123)	a 60 ± 1.85 (54 – 67) **(-A 50)	a 35.33 ± 1.82 (30 – 42) **(-A 70)	ac 179.33 ± 2.04 (173 – 187) **(+A 49) (+C 407)	ac 207.33 ± 2.11 (201 – 214) **(+A 74) (+C 486)
KIDNEY	104.83 ± 1.35 (100 – 109)	a 79.83 ± 1.74 (74 – 86) **(-A 23)	a 14.67 ± 1.28 (10 – 19) **(-A 86)	ac 159.17 ± 1.7 (153 – 165) **(+A 51) (+C 985)	ac 194.83 ± 1.94 (190 – 203) **(+A 85) (+C 1228)
BONE MARROW	110 ± 2.65 (101– 118)	a 65.17 ± 2.04 (60 – 73) **(-A 40)	a 35 ± 1.48 (31 – 41) **(-A 68)	ac 130.33 ± 1.94 (124 – 138) **(+A 18) (+C 272)	ac 179.67 ± 2.43 (171 – 188) **(+A 63) (+C 413)
F is significant at ** P < 0. 01, CD is significant at P < 0.05					

a, c significantly different from the group A and C.

Figures in parentheses are % of increase over control (+A), decrease (-A), malignant group (+C), (-C).

BRH_2 treated spleen, kidney and bone marrow on 20^{th} and 35^{th} day then control is yet lower than the DAL implanted (Group C) spleen, kidney and bone marrow.

Selenium level in different organs of mice under different treatment categories has been depicted in the ***table 6.3***. The Se concentration in the liver, spleen, kidney and bone marrow on both the10th & 20th day of DAL implanted mice were found to be significantly depleted ($p < 0.01$). On 20^{th} day the depletion was higher i.e. in case of liver (– 40%), spleen (– 70.6%), kidney (– 86%) and bone marrow (– 68.2%) respectively in comparison to the normal control.

The lymphoma implanted BRH_2 treated C_3H/He mice liver, spleen, kidney and bone marrow on the 20^{th} & 35^{th} day ($p < 0.01$) exhibited significantly increased level of Se over the normal control. The highest level of this elevation (120%) was observed in the liver of this series of treatment on the 35^{th} day.

Discussion:

Element profiles in biological system play significant role and hence considered useful as diagnostic markers in monitoring the pathological state. The quantitative variations of certain elements such as Cu, Zn & Se in

various tissues of C_3H/He mice, received lymphoma alone, and lymphoma with BRH_2 are depicted in the ***table 6.1, 6.2 and 6.3*** ; ***Figure 6.1, 6.2 and 6.3.***

The depletion of tissue copper in malignancy either in chemically induced or in implanted animals was earlier demonstrated (Lal et al., 1989; Ranade et al., 1989). The depleted level of copper in the uterine cancer tissue of man was described by Zhong et al. (1999). In support of the depleted Cu level, Lal et al. (1989) reported reduced amount of copper in cancerous esophagus of human being. The marginally depleted level of copper in the malignant kidney was observed by Margalioth et al. (1983) and Danielson and Steinnes (1970). Marginal fall of Cu in malignant kidney and colorectal tumor was earlier reported (Margalioth et al., 1983; Arriola et al., 1999).

However, Miyakawa (1990) observed some variation of erythrocyte copper concentration within normal range in patients having leukemia and lymphoma. Santoliquido et al. (1976) also observed no significant difference of copper values in the carcinoma of breast. Salkie et al. (1980) also observed no significant differences in the levels of Cu and Zn in smooth muscle of uteri with or without leiomyoma. Several of the first studies reported increased serum levels of Cu in patients with malignant tumors of various histotype and in different phases of the diseases (Gray, et al., 1982; Capel et al., 1982; Gozda et al., 1982). The depletion of copper level of malignant liver, spleen and kidney observed in this investigation might be

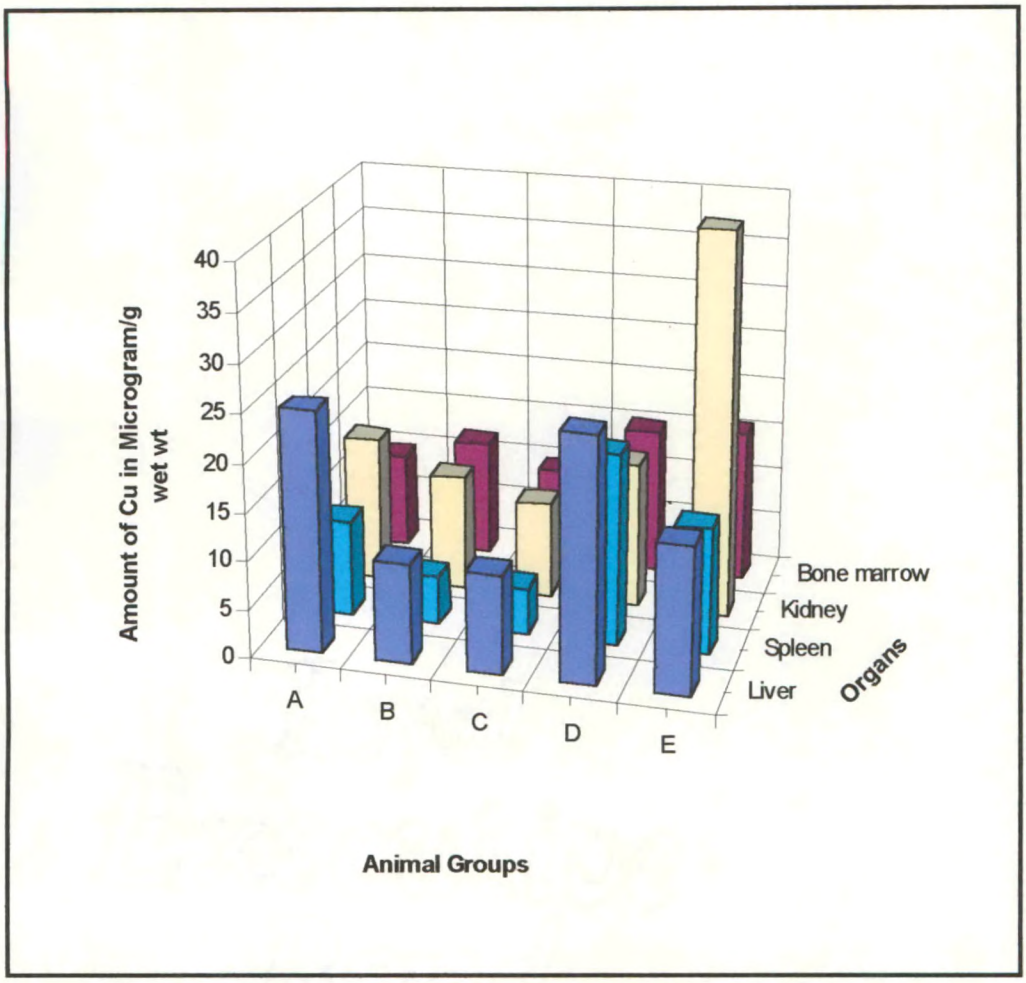

Figure 6.1: Profiles of Copper Concentration in diffarent organs of different groups of Mice.

A = Control, B & C = DAL implanted on 10th & 20th day,

D & E = DAL + BRH_2 treated on 20th & 35th day

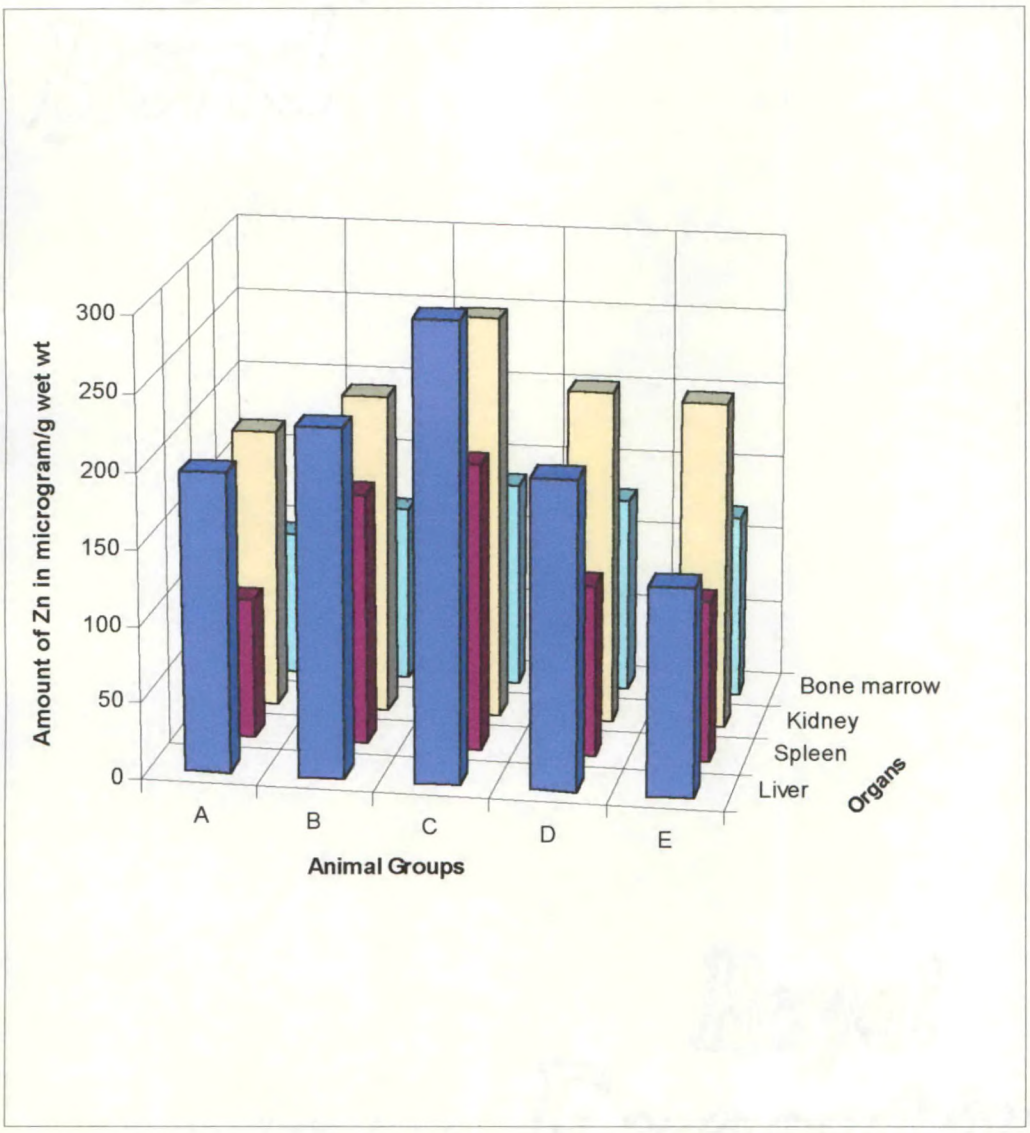

Figure 6.2: Profiles of Zinc Concentration in diffarent organs of different groups of Mice.

A = Control , B & C = DAL implanted on 10th & 20th day,

D & E = DAL + BRH_2 treated on 20th & 35th day

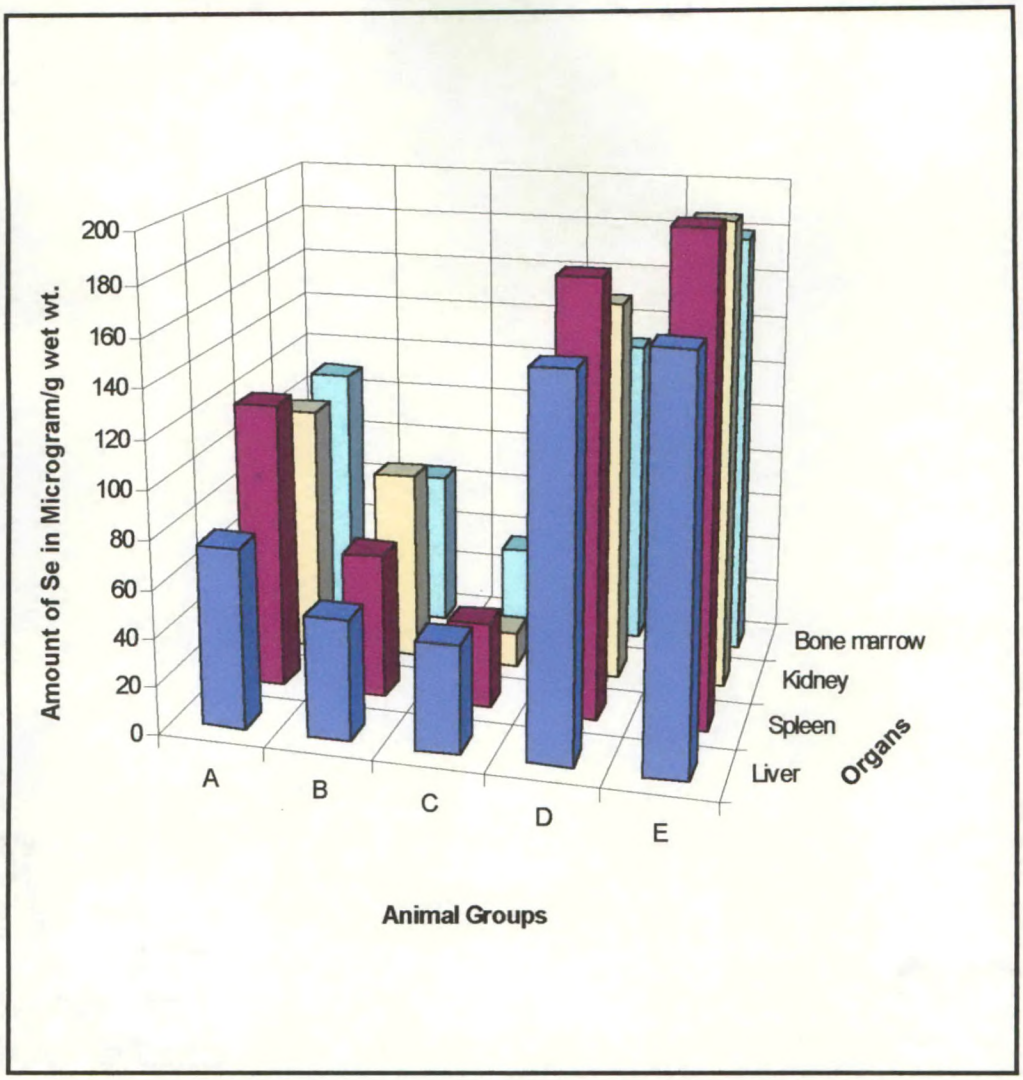

Figure 6.3: Profiles of Selenium Concentration in diffarent organs of different groups of Mice.

A = Control, B & C = DAL implanted on 10th & 20th day,

D & E = DAL + BRH_2 treated on 20th & 35th day

due to higher rate of copper excretion. Karchioglu et al. (1978) suggested that in the malignant kidney, normal reabsorption, function and the enzymatic activity of the tubular cells have been altered as well as the structure of its membrane. Therefore, it is speculated that during the neoplastic growth, the tubule cells loose their anatomical access to the glomerular filtrate and consequently the intake of the metal by renal absorption becomes impossible. Kitaura et al. (1999) suggested that renal tubular accumulation of Cu is the major cause of spontaneous renal carcinogenesis in Long Evans Cinnamon rats. Piccinini et al. (1996) reported that reduction of hair Cu level in breast and lung cancer compared to control. However, the results for copper concentration of these tissues of the present study are not in general agreement with the values obtained by other investigators for different tissues (De Jeorge et al., 1965; Mulay et al., 1971; Rizk and Sky-Peck, 1984; Jendryczko et al., 1986; Ranade et al., 1989).

The depletion of Cu (35%) in the BRH_2 treated malignant liver on the 35^{th} day ***(Table – 6.1)*** might be due to the altered permeability of the hepatic cell resulting in the enhanced excretion of copper, since, copper (II) dimethylglyoxime chelate shows coordination and mutual hydrogen bonding between the two bound ligands mask the hydrophilic oxime group and promotes its permeability through the cell membrane which has been found to inhibit the tumor growth of the animal with Ehrlich Ascites Tumor and Sarcoma 180 (Das, 1989).

The elevation of Cu level in the spleen, kidney and bone marrow in the BRH_2 exposed DAL by implanted mice probably be associated with the Cu present in the BRH_2 complex. Treating normal rats with weekly Cu injections significantly increased the Cu content of liver and kidney with no effect on intestinal Cu concentration (Irato et al., 1996). Lumme et al. (1984) found that presence of the hydrophilic groups in 2, 4 – dihydroxybenzaldoxime on benzene ring may make the metal complexes more soluble in aqueous solution thereby making them more suitable in anticancer preparation. The strong inhibiting power of the transplanar metal salicylaldoximates has been claimed due to their easy permeability towards the cell membrane and their unpaired 3d electrons. Trans – bis – (salicylaldoximato) metal (II) chelate resemble to vitamin B_6. It is well known that the vitamin B_6 antagonist e.g., 4 – deoxypyridoxime bears the antineoplastic activity and therefore, it is proposed that the antagonistic character of trans – bis – (salicylaldoximato) metal (II) chelate and especially that of the copper (II) chelate to vitamin B_6 is, at least partly responsible for the antineoplastic activity (Lumme et al., 1984). Copper – 2 – keto – 3 – ethoxybutyraldehyde bis – (thiosemicarbazone) is generally represented as "CuKTS' and the corresponding ligand as H_2KTS, which are effective against different types of tumors. The ligands H_2KTS released from the complex remains in equilibrium with the extra cellular medium but all of the copper remains in the cell. The reduction of Cu (II) KTS by thiol compounds (RSH) has been considered as the prime reaction lead to the antineoplastic activity. Analyzing the homogenates, after reaction, it has been found that most of

the copper is present as Cu (I) within the cell. The intracellular concentration of the thiols has been substantially decreased (Petering, 1980). Haratake et al. (1985) observed excessive copper accumulation in minute hepatoma suggesting the view that higher levels of copper in cancer cells was aggregated lysosomal metallothionine and that it might not be carcinogenic but stored by an altered metabolism of Cu and Cu – binding protein with the neoplastic transformation. Therefore, this might be a reason for the accumulation of copper in the D & E groups of spleen, kidney and bone marrow due to the altered metabolism of Cu and Cu – binding proteins, in the BRH_2 treated mice.

The exact mechanism of hypercupremia in malignancy is not clear. It is possible that tumors act by changing the capacity of the intestinal mucosa to absorb copper (Campbell et al. 1981). Alternatively, the increased copper levels may be a defense against tumors, as copper can bring back the growth of tumors in vitro (Petering, 1978) or inhibit their development in vivo (Linder, 1977).

The Zn concentrations in all the tissues i.e. liver, spleen, kidney and bone marrow were found to be significantly high in the DAL implanted mice over the control. The enhancement of tissue Zn concentration in various forms of malignancy was earlier demonstrated by different workers (Schicha et al. 1972; Schwartz et al. 1974; Chavpil, 1976; Santoliquido et al., 1976; Rizk and Sky – Peck, 1984; Muller et al., 1988). Earlier Koch and Smith (1956)

found a 4 – 7 fold increase in the level of zinc in papillary adenocarcinoma and reticulosarcoma. Petering et al. (1967), and Mc Quitty et al. (1970) observed that Zn is involved in the tumor growth and development of neoplastic transformation. Zaichick et al. (1995) observed elevated Zn level in malignant paranodular thyroid tissue in comparison to benign paranodular thyroid tissue, but significantly lower level in malignant thyroid nodules in comparison to benign thyroid nodule. Jayasurya et al. (2000) observed that tissue Zn levels were higher in nasopharyngeal cancer as compared with benign nasopharyngeal tissues (4.8 ± 0.461 versus 2.889 ± 0.4045 µg / g dry wt. Tissue) respectively. The mean tissue Zn level in nasopharyngeal tissue was 1.7 times higher than that in benign nasopharyngeal tissue, concurring with the finding that zinc levels are generally higher in cancerous tissues (Griffith et al., 1973; Rizk and Sky – Peck, 1983; Jin et al. 1999). Gouget et al. (2000) observed that Zn concentration is higher in both N – myc amplified cell lines (IMR – 32 and IGR – N – 91) than in non- amplified cells (SK – N – SH). Cu and Zn contents in both SK – N – SH and IGR – N – 91 tumor cells do not present significant differences compared to monolayers of cultured neuroblasts (Gouget et al., 2001).

Piccinini et al. (1996) observed that the Zn mean values in breast and lung cancer were significantly higher than in controls in both the samples. But in other works, including Piccinini et al. (1996), neither serum Zn nor the Zn/Cu ratio was able to discriminate between controls or patients with breast cancer (Garofalo et al., 1980). Recently, Geraki et al. (2004)

observed that Cu and Zn concentration in cancerous breast tissue sample is higher then the healthy tissue. Arriola et al. (1999) reported insignificant elevation of Zn in colorectal tumor tissue in comparison to normal tissue. Carvalho and Marques (2001) have observed high levels of Zn in the kidney tissue of liver cirrhosis patients. Tapper et al. (1987) have measured the elemental distribution in human brain tissue and observed higher levels of Zn in the tumor tissue compared with those in normal tissue.

The lower levels of Zn observed in the cancer tissue of stomach (Reddy et al., 2003) support the observation of Poswillo and Cohen (1971) and Cherkasova (1969) which states that the presence of Zn will inhibit the tumor growth. These conflicting observations suggest that the role of Zn in different organs may be different which supports the observations of Uda et al. (1987). Earlier Olson et al. (1954) also reported significant increase in Zn, Cu and Fe in patients with acute lymphatic leukemia. Melanoma cells take up substantial amount of ^{65}Zn in contrast to normal uveal pigment cells (O' Rourke et al., 1957; Borovansky et al., 1980). From the analysis of metal content of DNA and RNA isolated from different tumor tissues (Sarcoma M – I, Walker 256 Carcinosarcoma etc.) of rat liver, trace metals like Zn, Co, Fe, etc. are found considerably higher than those found in DNA and RNA of normal ones (Das, 1989).

Zhong et al. (1999) explained that concentration of Zn in human uterine cancer tissue was depleted in comparison to normal tissue. An association

Table: 6.4: Correlation coefficient (simple) among various elements in liver of control, DAL implanted (after 20 days), and BRH_2 treated (after 35 days) groups of mice.

Groups	Elements	Cu	Zn	Type of Relation
Control (A)	Zn	-0.95125*		Negative correlation
	Se	0.42361	-0.39136	between Cu & Zn
DAL implanted	Zn	-0.36847		
on 20th day (C)	Se	0.51036	-0.46974	
DAL + BRH2	Zn	0.52779		
on 35th day (E)	Se	0.02957	0.70255	

Table: 6.5: Correlation coefficient (simple) among various elements in spleen of control, DAL implanted (after 20 days), and BRH_2 treated (after 35 days) groups of mice.

Groups	Elements	Cu	Zn	Type of Relation
Control (A)	Zn	-0.88378*		Negative correlation
	Se	0.1527	0.26069	between Cu & Zn
DAL implanted	Zn	0.07537		
on 20th day (C)	Se	0.50791	0.6222	
DAL + BRH_2	Zn	-0.9048*		Negative correlation
on 35th day (E)	Se	0.35048	-0.39811	between Cu & Zn

*** indicate significant correlation coefficient at 0.05 level of probability.**

N = 6, Critical value (0.05) = 0.811

Table: 6.6: Correlation coefficient (simple) among various elements in kidney of control, DAL implanted (after 20 days), and BRH_2 treated (after 35 days) groups of mice.

Groups	Elements	Cu	Zn	Type of Relation
Control (A)	Zn	-0.07567		
	Se	0.48518	0.18476	
DAL implanted on 20th day (C)	Zn	0.10677		Negative correlation
	Se	0.2574	-0.87964*	between Zn & Se
DAL + BRH_2 on 35th day (E)	Zn	-0.5226		
	Se	0.22375	-0.70204	

Table: 6.7: Correlation coefficient (simple) among various elements in bone - marrow of control, DAL implanted (after 20 days), and BRH_2 treated (after 35 days) groups of mice.

Groups	Elements	Cu	Zn	Type of Relation
Control (A)	Zn	-0.39941		
	Se	-0.06337	-0.15242	
DAL implanted on 20th day (C)	Zn	0.10142		Positive correlation
	Se	-0.08598	0.87329*	between Zn & Se
DAL + BRH_2 on 35th day (E)	Zn	0.413931		
	Se	-0.14686	-0.1304	

*** Indicate significant correlation coefficient at 0.05 level of probability.**

N = 6, Critical value (0.05) = 0.811

has recently been reported between low Zn level and various types of immune deficiencies in subjects with lung cancer (WHO, Geneva, 1987; Crea et al., 1990). Slightly elevated levels of Zn were observed in the cancer tissue of kidney while definite low levels of Zn were observed in the cancer tissue of stomach (Reddy et al., 2003). Margalioth et al. (1983) observed no increase or decrease of overall Zn concentration in the malignant samples of female reproductive organs, compared to non-malignant tissues. Miyakawa (1990) also observed erythrocyte Zn concentration around the normal level in patients with leukemia and lymphoma. Uda et al. (1987) have estimated a deficiency of Zn in the cancer tissue of kidney while an opposite trend was observed in testicular cancer tissue. This suggests that the deficiency or excess of trace elemental concentrations in the cancerous tissues of different organs may be different. Kopanski et al. (2003) observed that the average concentration of Mg and Zn in the blood serum was lower in non-melanoma skin cancer (NMC) patients compared to control group and reference group patients. Prasad et al. (1997) found that plasma Zn and Cu levels in cancer patients were within the normal ranges, but lymphocyte and granulocyte Zn levels were significantly decreased in 53% of cancer subjects. Marginal Zn deficiency was reported to induce copper toxicities by reducing the Zn – Cu interrelationship (Johnson et al., 1966; Margalioth et al., 1983). Hypozincemia especially in the lymphoproliferative malignancies has also been reported (Koslowski et al., 1967).

It is interesting to note that the Zn concentration in tumor DNA varies slightly during the course of the disease and it suggests that Zn is urgently needed for the fast dividing cells characterized by a higher level of DNA synthesis. Hence, it is proposed that the removal of Zn from the nucleic acid would lead to retard the DNA synthesis and as a result, multiplication of the tumor cell will be arrested. In different types of animal cancer, dietary deficiency of Zn inhibits the tumor growth of L 1210 leukemia, P 388 leukemia etc. (Das, 1989). However, the tumor growth inhibitive property of Zn deficient diet can be rationalized from the stand point of the biological roles of Zn in nucleic acid and protein biosynthesis. (Issaq, 1980). Zn depletion after introduction of BRH_2 in C_3 H / He mice on 20^{th} and 35^{th} day ***(Table-6.2)*** possibly be seen as an detention of inhibitory effect on the DNA synthesis in the tissues. This provides an insight into the potentiality of BRH_2 in terms of its anticancerous activities. However, this characterization is very much required. It has been established that Zn occurs as a coenzyme of about 80 enzymes. DNA polymerase and RNA polymerase are both Zn containing metaloenzymes and these are intimately involved in nucleic acid metabolism. For structural confirmation of the nucleic acids, Zn is also an indispensable integral component. It is also essential for the amino acid utilization. As a matter of fact, most of the events of the cycle of cell division requires Zn. For the fast dividing malignant tissues, they may have a particular demand of Zn as a nutrient, hence, such tissues being deprived of this essential nutrient will not grow and the tumor growth may be inhibited when the patients are maintained on Zn deficient diet.

Since abnormal serum levels of Cu and Zn have generally been encountered in various cancers, serum Cu / Zn ratio has been recommended by many workers as a better prognostic marker than the individual levels of either of the two elements (Fisher, et al., 1976; Inutsuka and Araki, 1978). Muley et al. (1971) and Jha et al. (1985) observed elevation of Cu / Zn ratio in the malignant state, which indicated possible lowering of enzymatic capacities as suggested by Jendryczko et al. (1986). In the present investigation the Cu / Zn ratios of different organs, under different treatment series were observed ***(Tables –6.4, 6.5, 6.6, 6.7)***. Similar observations of the higher level of serum Cu / Zn ratio in oral carcinoma (Jha et al., 1985), the tissue Cu / Zn ratio in different malignant organs of this investigations were found to be higher except in the liver tissue. The highest elevation of Cu / Zn ratio was found in kidney tissue. Although a few investigators have observed an increased serum Cu / Zn ratio in head and neck cancer patients, it appears that this is a marker of an advanced and active cancer (Garofalo et al., 1980; Toke and Dhamne, 1990).

Zhong et al. (1999) observed extremely low-concentration of Se, Zn etc, in uterine cancer tissues and in chronic cervicitis tissues. Several of the first studies reported low serum levels of Se (Shamberger, et al., 1973; Burk, 1986) in patients with malignant tumors of various histotype and in different phases of the disease. In breast cancer, Se concentration in hair was found significantly reduced (Piccinni et al., 1996). Some case control studies have documented significantly lower serum selenium in breast cancer cases than

in controls (Mc Connell et al., 1980; Schrauzer et al., 1985 and Chaitchik et al., 1988) and others have found no such association (Meyer et al., 1987; Van't Veer et al., 1990 and van den Brandt et al., 1994). In a study on a group of patients with early stage, the serum concentration of Se was found to be reduced, and this datum, vary significantly, has been proposed as an additional noninvasive parameter for the clinical assessment of this malignant disease (Krsnjavi et al., 1990). A similar finding had already been pointed out only in patients with advanced breast cancer (Mayer and Verreault, 1987).

Depleted level of Se after DAL implantation in mice was observed in this investigation, which clearly suggests the metastasis in tissues. But the elevation of Se after BRH_2 treatment offers the (+ ve) role of this particular element. An inverse association between Se status and the risk of lung cancer was reported in prospective studies (Issel et al., 1981 and van den Brandt et al., 1993) and a direct correlation between Se status and good prognosis in clinical studies (Rosof and Spencer, 1965). Epidemiological studies have suggested that decreased selenium status in human is associated with increased risk of cancer (Combs et al., 1986). Prospective cohort studies in eastern Finland (Salonen et al., 1984) the USA (Willett et al., 1983; Helzlsouer et al., 1989), and in the Netherlands (Kok et al., 1987) have shown mean prediagnostic serum Se level to be significantly lower in cancer cases than in controls. In various epidemiological studies, associated between low selenium levels with an increased rise of cancer

incidence was described by several authors (Willet et al., 1983; Salonen et al., 1984; Salonen et al., 1985; Clark 1985; Menkes, 1986; Newberne et al., 1986; Coates et al., 1988; Iyengar and Woittiez, 1988; Knekt et al., 1988; Ringstad et al., 1988; Knekt et al., 1990; Pawlowicz et al., 1991; Turan and Delibasi, 1992; Pawlowicz et al., 1993; Torun et al., 1995). Zaichick et al. (1995) observed somewhat lower concentration of Se in malignant tissues from that of the standard. Kvicala et al. (1992) found that the selenium content was statistically lower both in thyroid carcinomas and adenomas than that present in the standard. In malignant thyroid nodules and paranodular thyroid tissues also characterized by a lower Se content when compared with the standard (Zaichick et al., 1995). Another correlation study showed that the serum Se of colon cancer cases was significantly lower than that in the general population (Caroli et al. 1994). A case control study (West et al., 1991) found that prostate cancer patients had lower plasma selenium than that in matched control, but the difference was not statistically significant.

Reddy et al. (2003) observed Se concentration in the tissue of the normal kidney, but no trace of this element was found in the tissue of carcinoma kidney. The same trend was also observed by Kwiatek et al. (1996) but no trace of Se was observed in normal as well as cancer tissues of stomach. A nested case control study of a large cohort found that higher toenail Se levels were associated with a reduced risk of advanced prostate cancer (Yoshizawa et al., 1998). An inverse association between dietary Se intake,

serum and toenail Se levels and cancer risk in human has been reported (Shamberger et al., 1976; Schrauzer et al., 1977; Clark et al., 1991 and Clark et al., 1996). This inverse association is also true in some animal studies (Thompson et al., 1980; and Medina et al., 1981; Combs et al., 1989) in ecologic and correlational studies (Shamberger et al., 1976; Schrauzer et al., 1977 and Clark et al., 1991) in case-control studies (Mc Connell et al., 1980; Schrauzer et al., 1985 ; Chaitchik et al., 1988; and Willett et al., 1991) and in some prospective studies (Wilett et al., 1983; Salonen et al., 1984; Salonen et al., 1985; Fex et al., 1987; Kok et al., 1987; Knekt et al., 1990 and Van den Brandt et al., 1993). One large cohort study of women in the Netherlands (Van Dokkum et al., 1989 and Van den Brandt et al., 1994) and two ecologic studies (Schrauzer et al., 1977 and Clark et al., 1991) reported an inverse association between Se and breast cancer. Ghadirian et al. (2000) observed higher toenail Se concentration, the strong risk reduction.

Animal and laboratory studies indicate the protective role of Se in the etiology of malignancies. Se has been suggested in many epidemiological studies and in other experiments to play an important preventive role in carcinogenetic process (Schrauzer, 1976). Recent studies have also suggested an antineoplastic activity by some forms of Se, in particular, the enhanced survival of Ehrlich ascites tumor-bearing mice treated with sodium selenite (Greder et al., 1980). In vitro experiments with a verity of tumor cell lines, mostly murine, have demonstrated growth inhibition by

some forms of Se (Milner et al., 1981). Earlier investigations in patients with glioma and intracranial maningioma showed that clinical observations were necessary to determine the usefulness of Se in cases of low-grade glioma (Philipov et al., 1990). These effects, however, generally occur at somewhat higher concentration of Se in the tumor and these were seen to exert much effect on tumor development. The mechanism appears to involve DNA cleavage and DNA replication inhibition by various Se compounds (Cox, 1984 and Weitberg et al., 1985). It has been demonstrated that Se at 3 – 5 μg/L in vivo was sufficient to inhibit some mammary tumors (Medina et al., 1981). A widely publicized chemoprevention study has shown that Se supplements can decrease the incidence of certain types of cancer (Clark et al., 1996). Se has also been shown to have an inhibitory effect on chemical (Schillaci et al., 1982 and Jacobs, 1983) and viral carcinogenesis (Milner, 1985); it modulates cellular proliferation (G_1 phase), both in normal and neoplastic cells (Le Boeuf et al., 1986) and regulates the expression of oncogenes c- fos and c – myc (Yu et al., 1990).

In previous works on lung cancer, a rise in serum Cu and a fall in Se and Zn levels are more consistently observed in patients than in control (Comstock et al., 1992). Increased levels of Se, Zn, and Cu and glutathione – peroxidase have been found in neoplastic tissue from the human breast, lung, and colon (Rizk et al., 1984; Siegers et al., 1984; Di Ilio et al., 1985; and Di Ilio et al., 1988). Reddy et al. (2003) observed that the level of Se is lower and the level of Zn is higher in cancer tissue of kidney than those in

the normal tissue; but the level of Cu in the cancer tissue of kidney is in agreement with those observed in the normal tissue while in case of stomach Cu and Zn are lower than those observed in the normal tissue. The observed high levels of Zn in the cancer tissue of kidney suggest that Zn is involved in the tumor growth and development of neoplastic transformation in kidney while the observed low levels of Zn in the cancinoma tissue of stomach suggests that Zn inhibits the growth of cancer in this organ. Dobrowolski et al. (2002) observed that Zn and Se are strongly decreased in the neoplastic mass while raising the concentrations of Zn and Se with progress of malignant disease. Hardell et al. (1994), made the same observation. Changes in Zn and Se concentrations in malignant disease are known from research on other cancers (Boffetta, 1993).

The serum selenium levels of healthy subjects and of lung cancer patients in the high lung cancer region were 0.088 ± 0.001 and 0.070 ± 0.013 ppm, respectively (Zhu Y et al., 1982). In a low lung cancer region they were 0.123 ± 0.002 ppm. Although several previous studies indicated lower serum or plasma Se levels in skin cancer patients than in non-cancer patients or healthy subjects. These are not necessarily conclusive as cancer patients may be low in Se for reasons unrelated to diet (Clark et al., 1982). One case control study, which does not suffer from this potential shortcoming, involved 240 cancer patients examined at the Wilson Dermatology clinic in Wilson, North Carolina, between 1974 and 1980. The odds ratio for the lowest versus the highest decile of plasma Se versus

current or past clinic controls was 4.39 or 5.81 respectively. Serum Se levels of the gastrointestinal tract cases were significantly lower than those of controls from the same set, matched for age, race, sex, and smoking history a case study (Willett et al., 1983). The mean value of serum Se in healthy persons was 106.5 μg/l (SD = 16.5 μg/l) and the mean value of Se levels in non-small cell lung cancer and adenocarcinoma patient groups with 54.4 μg / l (SD = 18.5 μg / l) was statically significantly decreased ($p < 0.0001$) (Mucke et al., 2000). A number of investigators reported an association between Se and breast cancer (Gupta et al., 1994; Sharma et al., 1994; Piccinini et al., 1996; Haung et al., 1999 and Cann et al., 2000). Others found no correlation (Hunter et al., 1990; Van den Brandt et al., 1994; Van't Veer et al., 1996; Dorgan et al., 1998; Mannisto et al., 2000). Epidemiological studies have suggested that low levels of Se are associated with a higher incidence of both lung and prostate cancer, but no significant difference in mean serum Se in lung cancer cases versus controls (11.91 μg/dl versus 11.77 μg/dl) or prostate cancer cases versus controls (11.48 μg / dl versus 11.43 μg / dl) [Goodman et al., 2001]. An inverse association between toenail Se and bladder cancer risk was most pronounced among ex-smokers (P – trend < 0.01) (Zeegers et al., 2002). Also, an inverse relationship between the Se and Zn in liver, kidney, spleen and bone marrow after introduction of BRH_2 on 20^{th} and 35^{th} day could be established in this investigation and may be used in the determination of Zn / Se ratio as well as bio marker, however, full details are necessary.

The coefficient correlation study among the element of different organs of the present study showed certain interrelationship between different element pairs (Cu/Zn, Cu/Se and Zn/Se). The normal liver maintains negative correlation between Cu and Zn ***(Table 6.4)***. The normal spleen showed highly significant negative correlation between the element pairs Cu and Zn, while 35th day DAL implanted BRH_2 treated displayed highly negative correlation between Cu and Zn ***(Table 6.5)***. The DAL implanted kidney exhibited negative correlation between Zn and Se (Table. 6.6). The correlation between Zn and Se in bone marrow showed highly positive correlation ***(Table. 6.7)***.

Summary:

Element profile of the control, DAL implanted and chemotherapy given liver, spleen, kidney and bone marrow of white mice was analysis by A. A. S.

1. The significant decrease of Cu level in DAL implanted tissues was enhanced after BRH_2 administration, however, higher than control except liver.
2. Zn concentration was increased significantly in DAL implanted tissues in comparison to control, however, decreased after BRH_2 treatment, yet higher than the control level except liver.

3. Se level in DAL implanted tissues appeared to be depleted significantly, however, enhanced after BRH_2 treatment, but the highest elevation was found in kidney than the normal control.
4. An inverse relationship between Zn and Se is possible after BRH_2 administration.