CHAPTER V

LACTIC DEHYDROGENASE ACTIVITY (LDH) AND ISOENZYME PATTERN BEFORE AND AFTER BRH_2 TREATMENT IN THE DALTON'S LYMPHOMA IMPLANTED LIVER, SPLEEN, KIDNEY AND BONE MARROW OF WHITE MICE

LACTIC DEHYDROGENASE ACTIVITY AFTER BRH_2 TREATMENT IN DALTON'S LYMPHOMA IMPLANTED MALIGNANT LIVER, KIDNEY, SPLEEN AND BONE MARROW OF WHITE MICE.

Lactic dehydrogenase (LDH) is a glycolytic enzyme and reversibly catalyzes the pyruvate to lactic acid (Malhotra et al., 1986; Schwatz, 1992; Schwatz, 1991; Bates et al., 1985; Finck et al., 1983; & Ravel, 1995). LDH and its isoenzymes are of clinical interest because their use as molecular marker in malignancy receives wide attention (Burgess et al., 1963; Kawakatsu et al., 1963; Dhawan et al., 1971; Hariharan, et al., 1977). Earlier investigations revealed increase of LDH activity in a number of transplanted tumors (Bailey et al., 1964, Hsich et al., 1955) as well as in different stages of malignancy (Hsich et al., 1955; Friend & Wroblewski, 1956; Riley & Wroblewski, 1960). The conditions frequently associated with elevated serum LDH level include malignant tumor, hemolytic or megaloblastic anemia, laboratory artifact haemolysis, acute myocardial infarction, and skeletal muscle damage etc. (Ravel, 1995). However, Dokov (1985) reported increased LDH activity in implanted carcino sarcoma. The increased activity of serum LDH in cancer patients is attributed to the release of enzymes from malignant cells (Malhotra et al., 1986). LDH activity was also noted in a variety of cancerous growth viz. Pancreatic carcinoma (Bardawill & Chang, 1963; Maity & Burma, 1973), carcinoma of gastro-intestinal tract, hepato-biliary cancer, bone cancer and breast cancer

(Rao et al., 1978; Ghool et al., 1980; Yasowardhana, 1986). In testicular carcinoma the serum LDH activity was 7 times higher than normal tissue (Kuroda et al., 1985). Serum levels of LDH are high in metastatic liver tumors and in other malignancies (Finck, 1983; Bates et al., 1985; Malhotra et al., 1986; Schwatz, 1991; Schwatz, 1992). Serum LDH levels are high in metastatic Nasopharyngeal carcinoma (Liaw et al., 1997). Elevation is most frequently encountered in patients with certain malignancies, such as leukemia, lymphoma, and malignant germ cell tumors (Bates et al., 1985; Malhotra et al., 1986; Taylor et al., 1986; Schwatz, 1991; Schwatz, 1992; Ravel, 1995). Faluk et al.(1972), reported that tissue and serum LDH acts as a marker in gastric carcinoma . LDH has been considered as tumor marker in the diagnosis of head and neck cancer of human (Singh et al., 1993) while some other workers termed LDH as bio indicator and bio-marker (Messey, 1971; Jorgensen, 1989).

Lactic dehydrogenase, a cytoplasmic enzyme of mitochondrial origin is also found in microsomal fraction. LDH is a zinc protein and Zn is an integral part of the protein. The enzyme activity is inhibited by oxalate and urea, strong alkaline solution, inorganic phosphate and carbonate ions (Vassault, 1981).

It exists in multiple molecular forms within a single tissue and is a tetramer composed of four sub units. There are two types of subunits being under the control of separate gene (Kline and Clayton, 1964) which combines in all possible combination to yield five functionally similar forms called isoenzymes (Wigert and Villee, 1964) which can individually be identified by electrophoretic zymogram, possesses different physiological functions which are affected by oxidative condition. The five isoenzymes are present almost in all cells paralleling their major glycolytic pathways (Goodfriend et al., 1965). Tissue in which aerobic pathways predominate exhibits a preponderance of LDH_1 and tissues in which anaerobic glycolytic pathways may assume significant importance shows preponderance of LDH_5. The relative distribution of the isoenzymes are tissue specific and in various diseases changes in serum LDH reflects the isoenzymic pattern of the effected tissues.

In various human and animal malignancies along with the increase in total LDH shift in the isoenzyme pattern towards mascular type with an increase in the percentage of LDH_4 and LDH_5 activity has been recorded (Giannoulaki et al., 1989). LDH_5 has been found to be aerobically active in various malignancies notably brain, lung, stomach, breast, kidney, and prostate (Heckl & Fogh, 1986; Shimamura & Igakki, 1990). However, inconsistent alteration of LDH_2, LDH_3, and LDH_4 in malignant prostate gland was observed by Oliver and associates (1970), while Ricerca et al. (1986) observed decrease of LDH_1 and increase in LDH_2, LDH_3 and LDH_4, as well as total serum LDH in granulolytic leukemia. Wang (1991) recorded decreased LDH_2, LDH_3 and enhanced LDH_4, LDH_5 in cervical carcinoma of mice. LDH isoenzyme(s) also varies significantly in different malignant group notably of LDH_4 and LDH_5 in the primary carcinoma of ovary (Kukuchi et al., 1991); increased LDH_1 and LDH_2 in oesophageal, prostatic, ovarian carcinoma (Ananthanarayan & Ramakrishnan, 1978) and significantly higher LDH_4 and LDH_5 and lower LDH_1, LDH_2, LDH_3 in the primary lung cancer (Balinsky and associates, 1984) have been reported.

Monitoring of LDH activity in varieties of cancers during chemotherapy with adriamycin, cisplatin, vincristine, mytomycin and cyclophosphamide was reported by Nakamura and Kitagawa (1985). They observed that LDH level presented parallel changes with tumor size. If the enzyme is increases persistently after administration of effective drugs death can be predicted. Hydroxanthine altered the ratio of lactate content in various tissues and

normalised the liver tissue isoenzyme spectra in tumor bearing rats (Valichko et al., 1981). Cisplatin based chemotherapy was used for prognostic implication of chemical characteristics (Von Eyben et al., 1992).

Although, no such reports are available in terms of LDH and its isoenzyme as marker in the prognostic assessment after the administration of Cu based drug, yet in present investigation the LDH has been employed as marker in mice after implantation of Dalton's Lymphoma.

Materials and methods:

The details of materials and methodology have been described in the chapter II. In short, the three sets of C_3H/He mice (age 8 – 10 weeks; 18 – 22 gm b.w.) were undertaken in this experiment.

CONTROL SET – I

Group A: Normal (C_3H/He) receives 0.2 ml 0.75% aqueous carboxymethyle cellulose solution.

MALIGNANT SET – II

Group B & C: C_3H/He Mice implanted with Ascite Dalton's lymphoma cell by injecting (i/p) 0.2 ml (1×10^7 cells), killed on 10^{th} & and 20^{th} day respectively.

CHEMOTHERAPY SET – III

Group D: BRH_2 (100 mg/ kg b.w./ on 1^{st} , 5^{th}, & 9^{th} day) i.p. as in group B sacrificed on 20^{th} day.

Group E: BRH_2 (100 mg/ kg b.w./ on 1^{st} , 5^{th}, & 9^{th} day) i.p. as in group B sacrificed on 35^{th} day.

Results:

The total LDH activity in the present experimental set up I - control set, II- Malignant, and III – chemotherapy given set are as follows ***(Fig. 5.1; Table 5.1)***. The enzyme activity is expressed as IU/gm wet tissue, mean ± SEM of 6 animals.

LIVER

CONTROL SET – I

A: The LDH activity of the control liver was 23. 66 ± 1.47 (21.5 – 25.2) IU

MALIGNANT SET – II

B: The liver of this group presented 18.8% increase in LDH level over the control (A). The estimated mean activity was 28.1 ± 1.59 (25.8 – 30.2) IU.

Table. 5.1: LDH activity (IU/gm of tissue, Mean ± SEM of 6 animals in the liver, spleen, kidney and bone marrow of white mice.

Groups / Organs	A	B	C	D	E
Liver	23.66±1.47	**a 28.1± 1.59 (+A 19)	**ab 31.09±2.41 (+A 31) (+B 10)	**abc 7.73±1.39 (-A 67) (-B 72) (-C 75)	**abc 6.7±1.05 (-A 72) (-B 76) (-C 78) (-D 13)
Spleen	14.29±1.83	14.63±1.68 (+A 2)	15.0±1.02 (+A 5) (+B 2)	**abc 7.78±1.38 (-A 46) (-B 47) (-C 48)	**abcd 5.52±1.23 (-A 61) (-B 62) (-C 63) (-D 29)
Kidney	14.37±2.13	**a 20.25±1.44 (+A 41)	**ab 22.44±1.78 (+A 56) (+B 11)	**abc 6.53±1.35 (-A 55) (-B 68) (-C 71)	**abc 6.36±1.16 (-A 56) (-B 69) (-C 72) (-D 3)
Bone marrow	8.35±0.67	8.48±0.85 (+A 2)	8.71±1.39 (+A 4) (+B 3)	8.49±0.68 (+A 2) (+B 0.12) (-C 3)	**abcd 6.88±0.66 (-A 18) (-B 19) (-C 21) (-D 19)

F is significant at ** P< 0.01, CD is significant at P<0.05.

A - Control; B & C - Lymphoma implanted mice sacrificed on 10^{th} and 20^{th} day; D & E - BRH_2 treated lymphoma-implanted mice sacrificed on 20^{th} and 35^{th} day.

Figures in parenthesis are % of increase over control (+A), decreased (-A), (+B) & (-B) 10^{th} day, (+C) & (-C) 20^{th} day malignant group and (-D) malignant group BRH_2 treated on 2oth day.

a,b,c,d significantly different from the group A,B,C,D respectively.

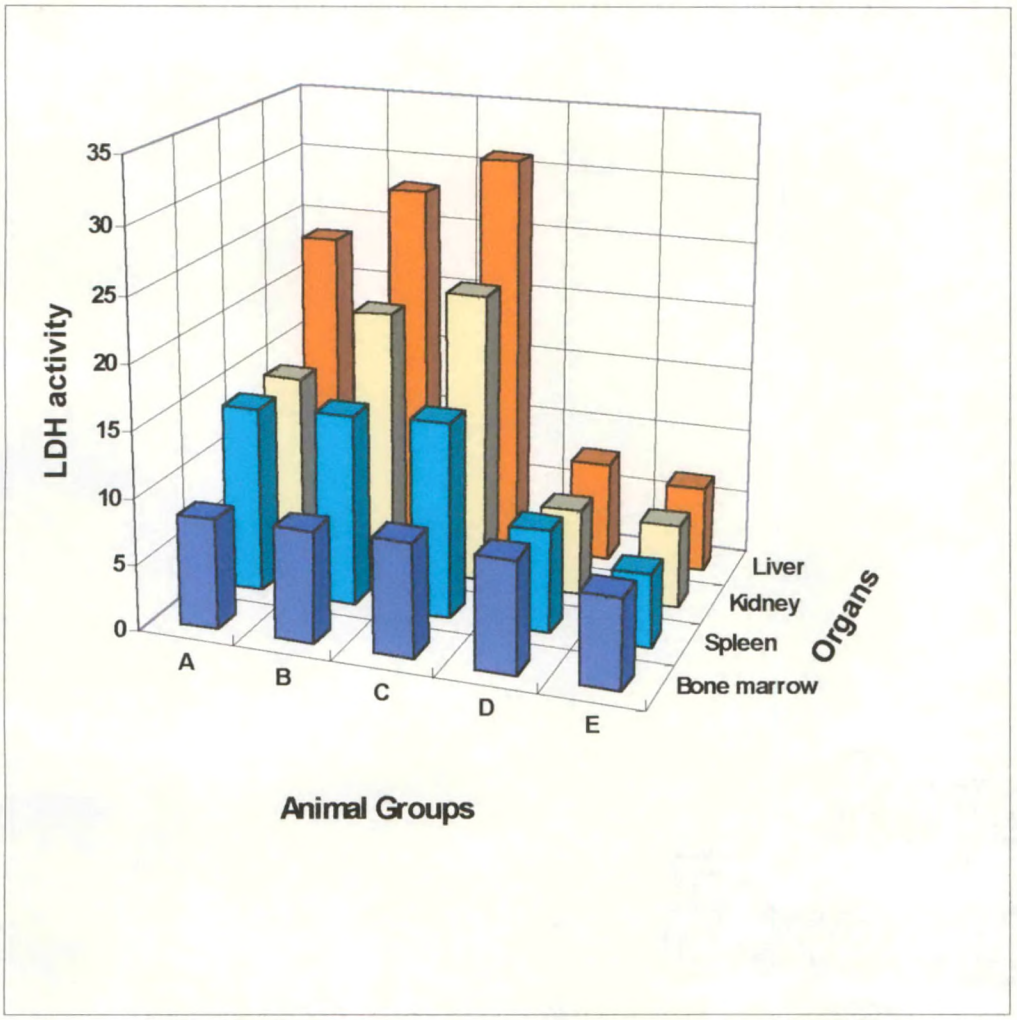


Figure 5.1: Profiles of LDH activity in diffarent organs of different groups of Mice.

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

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

C: There was 31.4% increase of LDH activity displayed by the liver of this group over the control (A) while the 10.6% increase over group B. The average activity was 31.09 ± 2.41 (28.8 – 34.9) IU.

CHEMOTHERAPY GIVEN SET – III

D: The total LDH activity was 7.73 ± 1.39 (6.2 – 10.0) IU. The activity was reduced by 67.33% compared to control (A) and the reduction of enzyme activity was 72.5% and 75.1% from the mean activity of group B and C.

E: The total LDH activity was 6.7 ± 1.05 (5.4 – 8.3) IU. The activity was reduced by 71.68% compared to Group A and the reduction of enzyme activity was 76.2%, 78.5% from the mean activity of group B and C.

SPLEEN

CONTROL SET – I

A: The total LDH activity in control spleen of mice was 14.29 ± 1.83 (11.9 – 16.5) IU.

MALIGNANT SET – II

B: The spleen showed mean LDH level 14.63 ± 1.68 (12.4 – 17.2) IU. The activity was found to be higher by 2.4% over the control.

C: The spleen LDH activity was increased by 2.5% compared to group B and 50% over the control (A). The total LDH activity was 15.0 ± 1.02 (13.8 – 16.5) IU.

D: The spleen presented significantly depleted LDH activity. The mean activity was 7.78 ± 1.38 (5.8 – 9.5) IU. The LDH activity was decreased by 45.56% compared to control (A) while the reduction of 46.8% and 48.1% from the B and C group was recorded respectively.

E: The spleen LDH activity of this group was 5.52 ± 1.23 (3.9 – 7.0) IU. The activity was found to be reduced by 61.37% from the control, 62.3% from group B; 63.2% from group C and 29.1% from group D.

KIDNEY

CONTROL SET – I

A: The mean LDH activity of kidney was 14.37 ± 2.13 (12.2 – 17.3) IU.

MALIGNANT SET – II

B: The LDH activity of this group was recorded to be significant. The average enzyme activity was 20.25 ± 1.44 (18.9 – 22.6) IU, which was 40.9% more than the control.

C: The mean kidney LDH activity was 22.44 ± 1.78 (20.3 – 25.2) IU with 56.2% more than the control (A). and 10.8% over the group B.

D: The total LDH activity in the BRH_2 treated mice kidney was 6.53 ± 1.35 (4.8 – 8.2) IU. This activity was found to be depleted by 54.6% from the control, 67.8% from the group B and 71% from the group C.

E: The total LDH activity in this group was 6.36 ± 1.16 (5.0 – 7.8) IU and was decreased by 55.7% than compared to control. The fall of the enzyme activity was 68.6%, 71.7% and 2.6% from group B, C & D respectively.

BONE MARROW

CONTROL SET – I

A: The total LDH activity in control bone marrow of mice was 8.35 ± 0.67 (7.5 – 9.3) IU.

MALIGNANT SET – II

B: The bone marrow LDH activity was 8.48 ± 0.85 (7.2 – 9.5) IU. The activity was found to be higher by 1.6% over the control.

C: The total LDH activity of the bone marrow was 8.71 ± 1.39 (7.0 – 10.5) IU. The activity was found to be higher by 4.3% and 2.7 % over the group A and B respectively.

D: The bone marrow LDH activity of this group was 8.49 ± 0.68 (7.5 – 9.2) IU. The activity was more than 1.7% and 0.12% over the group A & B, but 2.6% reduction was noted from group C.

E: The bone marrow LDH activity of this group was 6.88 ± 0.66 (6.0 – 7.8) IU. The LDH activity was significantly reduced by 17.6%, 18.9%, 21.0% and 19% from the group A, B, C, and D respectively.

Isoenzyme study:

The prepared gel was placed under VDS – image master for scanning and the pixel intensity was calculated from normal control, malignant (B and C) and BRH_2 treated groups (D and E). Three prominent bands in the control liver i.e. LDH_2 (Pi 125), LDH_3 (Pi 140), LDH_4 (Pi 95) along with two feeble bands LDH_1 (Pi 80) & LDH_5 (Pi 25) were recorded. The 20^{th} day lymphoma implanted liver presented prominent bands of LDH_1 (Pi 105), LDH_2 (Pi 125), LDH_3 (Pi 145), LDH_4 (Pi 110), LDH_5 (Pi 95). The 20^{th} day lymphoma implanted BRH_2 treated liver displayed prominent bands LDH_2 (Pi 120), LDH_3 (Pi 110) and others were feeble LDH_1 (Pi 60), LDH_4 (Pi 55) & LDH_5 (Pi

Table: 5.2: Pixel intensity of LDH isoenzymes in different groups of liver of C_3H/He mice

Bands / Groups	1	2	3	4	5
Control	80	125	140	95	25
DAL 20th day	105	125	145	110	95
DAL + BRH2 20 th day	60	120	110	55	60
DAL + BRH2 35th day	100	100	90		55

Table: 5.3: Pixel intensity of LDH isoenzymes in different groups of spleen of C_3H/He mice

Bands / Groups	1	2	3	4	5
Control	125	160	125	190	75
DAL 20th day	155	175	150	130	25
DAL + BRH2 20 th day	80	55	75	140	145
DAL + BRH2 35th day	150	80	125	140	80

Table: 5.4: Pixel intensity of LDH isoenzymes in different groups of kidney of C_3H/He mice

Bands / Groups	1	2	3	4	5
Control	75	90	95	40	45
DAL 20th day	110	145	150		85
DAL + BRH2 20th day	80	140	110	100	55
DAL + BRH2 35th day	55	70	85	105	60

Table: 5.5: Pixel intensity of LDH isoenzymes in different groups of bone marrow of C_3H/He mice

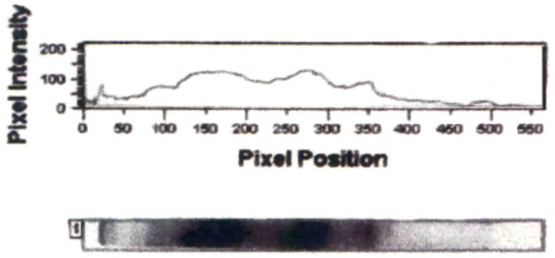
Bands / Groups	1	2	3	4	5
Control	120	170	125	55	50
DAL 20th day	140	175	125	55	60
DAL + BRH2 20th day	110	170	75	65	150
DAL + BRH2 35th day	90		140	50	60

60). The 35th day lymphoma implanted BRH_2 treated liver presented sharp bands of LDH_1 (Pi 100), LDH_2 (Pi 100), LDH_3 (Pi 90), and a weak band LDH_5 (Pi 55). ***(Figure. 5.2, Table. 5.2)***.

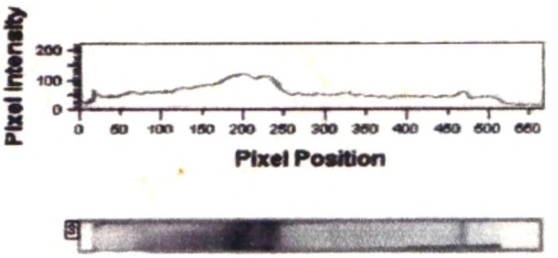
The control spleen showed LDH_1 (Pi 125), LDH_2 (Pi 160), LDH_3 (Pi 125), LDH_4 (Pi 190) and LDH_5 (Pi 75). The 20th day malignant spleen displayed LDH_1 (Pi 155), LDH_2 (Pi 175), LDH_3 (Pi 150), LDH_4 (Pi 130) and LDH_5 (Pi 25). The 20th day malignant and BRH_2 treated spleen displayed two prominent bands and LDH_4 (Pi 140), LDH_5 (Pi 145) and three feeble bands LDH_1 (Pi 80), LDH_2 (Pi 55) and LDH_3 (Pi 75). The 35th day malignant and BRH_2 treated spleen showed three prominent bands LDH_1 (Pi 150), LDH_3 (Pi 125), LDH_4 (Pi 140), and two feeble bands LDH_2 (Pi 80), and LDH_5 (Pi 80). ***(Fig.5.3, Table 5.3)***

Control kidney showed two prominent isoenzyme bands, namely LDH_2 (Pi 90), and LDH_3 (Pi 95), and three feeble bands LDH_1 (Pi 75), LDH_4 (Pi 40), and LDH_5 (Pi 45). The 20th day lymphoma implanted kidney projected three prominent bands LDH_1 (Pi 110), LDH_2 (Pi 145), and LDH_3 (Pi 150), and one feeble band for LDH_5 (Pi 85). The 20th day lymphoma implanted BRH_2 treated kidney displayed three prominent bands LDH_2 (Pi 140), LDH_3 (Pi 110), and LDH_4 (Pi 100), and two feeble bands LDH_1 (Pi 80), and LDH_5 (Pi 55). The 35th day lymphoma implanted BRH_2 treated kidney presented two distinct bands LDH_3 (Pi 85), and LDH_4 (Pi 105), and other were LDH_1 (Pi 55), LDH_2 (Pi 70), and LDH_5 (Pi 60) as feeble bands. ***(Fig 5.4, Table 5.4)***

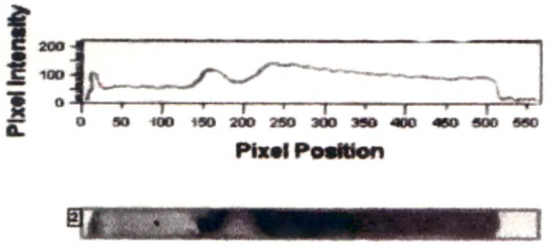
The control bone marrow showed LDH_1 (Pi 120), LDH_2 (Pi 170) and LDH_3 (Pi 125) with high pixel intensity and with two low intensities for LDH_4 (Pi 55) and LDH_5 (Pi 50). The 20^{th} day lymphoma implanted bone marrow displayed very prominent peaks for LDH_1 (Pi 140), LDH_2 (Pi 175), and LDH_3 (Pi 125) while low peak for LDH_4 (Pi 55) and LDH_5 (Pi 60). The 20^{th} day lymphoma implanted BRH_2 treated bone marrow showed prominent peaks for LDH_1 (Pi 110), LDH_2 (Pi 170) and LDH_5 (Pi 150) whereas peaks for LDH_3 (Pi 75) and LDH_4 (Pi 65) were detected. The 35^{th} day lymphoma implanted BRH_2 treated bone marrow presented LDH_1 (Pi 90) and LDH_3 (Pi 140) with high and others LDH_4 (Pi 50) and LDH_5 (Pi 60) exhibited low intensity. ***(Fig 5.5, Table 5.5).***



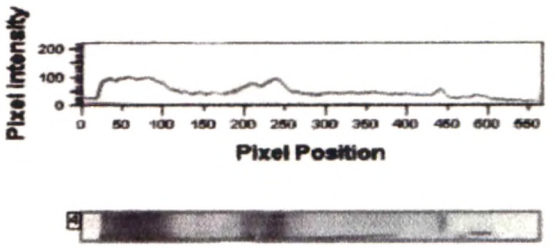
Lane 1 - Control



Lane 3 - DAL +BRH_2 (after 20 days)

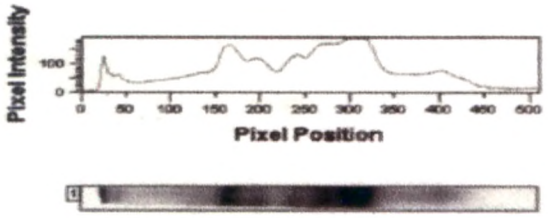


Lane 2 - DAL implated (after 20 days)

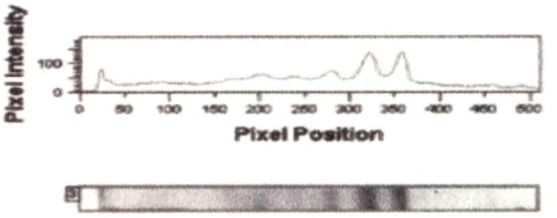


Lane 4 - DAL +BRH_2 (after 35 days)

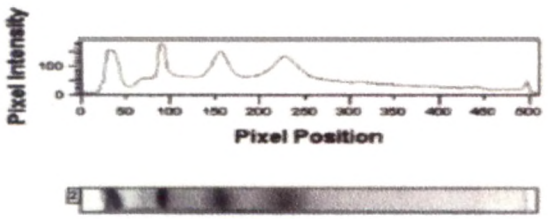
Fig: 5:2 LDH isoenzyme pixel intensity of Liver of white mice.



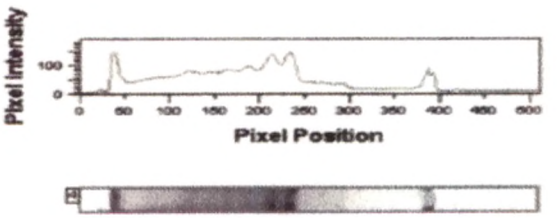
Lane 1 - Control



Lane 3 - DAL +BRH_2 (after 20 days)

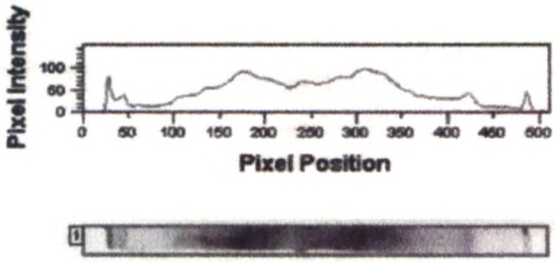


Lane 2 - DAL implated (after 20 days)

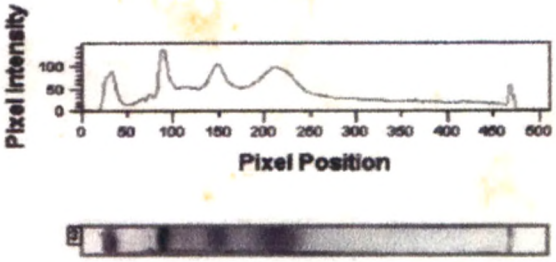


Lane 4 - DAL +BRH_2 (after 35 days)

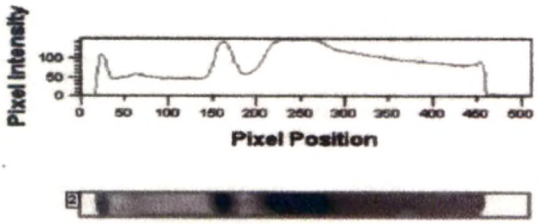
Fig: 5:3 LDH isoenzyme pixel intensity of Spleen of white mice.



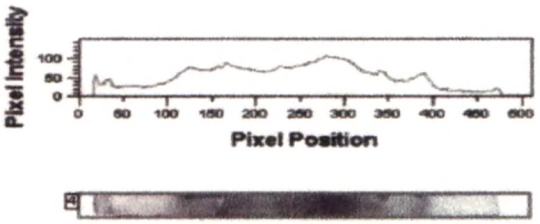
Lane 1 - Control



Lane 3 - DAL +BRH_2 (after 20 days)

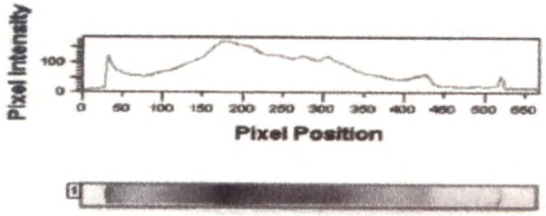


Lane 2 - DAL implated (after 20 days)

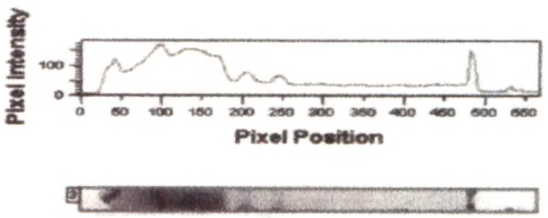


Lane 4 - DAL +BRH_2 (after 35 days)

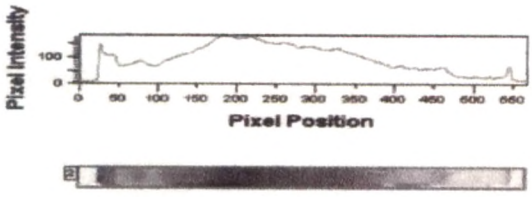
Fig: 5:4 LDH isoenzyme pixel intensity of Kidney of white mice.



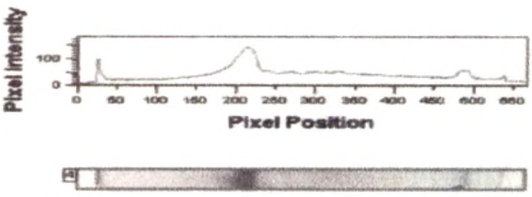
Lane 1 - Control



Lane 3 - DAL +BRH_2 (after 20 days)



Lane 2 - DAL implated (after 20 days)



Lane 4 - DAL +BRH_2 (after 35 days)

Fig: 5:5 LDH isoenzyme pixel intensity of Bone Marrow of white mice.

Discussion:

LDH activity in lymphoma implanted malignant liver, spleen, kidney and bone marrow of mice was found to enhance on the 10^{th} day and the highest activity was being recorded on the 20^{th} day of malignant mice over their respective control. On the other hand lymphoma implanted BRH_2 treated organs of mice the LDH activity was found to be depleted significantly from the control and lymphoma implanted group on the 35^{th} day. ANOVA presented significant LDH variation in all the cases ($P < 0.01$). Association of enhanced LDH activity was noted in the gastro-intestinal tract, bone cancer, breast cancer of many experimental animals and human being (Maity & Burma, 1973; Rao et al., 1978; Ghool et al., 1980; Yasowardhana, 1986). The progressive increase of LDH activity in different carcinoma and in different stages has already been reported (Faluk et al., 1972; Kuroda, et al., 1985). Elevated serum LDH levels are usually seen in metastatic liver tumor (Finck et al., 1983; Bates et al., 1985; Schwatz, 1991; Schwatz, 1992 and Ravel, 1995). Serum LDH levels are high in metastatic Naso-pharyngeal carcinoma (Hoch-Ligeti et al., 1976; Micheau et al., 1987 & Cvitkovic et al., 1991). Marked increase in serum LDH in carcinoma of the pancreas was investigated earlier (Bardawill and Chang, 1963). The enhanced level of LDH in Dalton's lymphoma implanted liver, spleen, kidney and bone marrow experimented on 20^{th} and 35^{th} day maintained identical trend and in conformity with the observation of Dokov (1985) that the increase in LDH activity in the methylcholanthrene induced implanted carcinosarcoma was not uniform. Earlier, Hoch Ligeti et al. (1964)

determined the LDH activity in nitrosamine induced carcinogenesis of rat and suggested that changes caused by carcinogen in the cytoplasmic protein molecule either directly or consecutive to alteration the template of nucleic acids might manifest the changes in the enzyme activities.

Earlier Singh et al. (1993) reported that poorly differentiated carcinoma had higher levels of serum LDH compared to moderately differentiated tumors. However, it could not be confirmed from the present findings, which demand further investigation. The consistent observation of lactic acid accumulation, even in the presence of oxygen has already been documented (Goldman et al., 1964). The LDH would be expected to play a critical role in this regard. Therefore, there is implication of a functional difference in this enzyme between malignant tumors and normal tissues.

Increase in anaerobic glycolysis predominantly in gastro-intestinal malignant tumors (Mc Beth and Bekesi, 1962) is the striking parallel with the present findings. Despite of graded increase in glycolytic activity of the malignant tissues with increasing histologic degree of malignancies in terms of mean values of LDH activity, the variability within each group is remarkable. These result, invariably suggest a relationship between the degree of malignancy and glycolysis as evident in the tissues of 35^{th} day DAL implanted mice.

DAL implanted BRH_2 treated mice of this investigation revealed that the LDH activity was remarkably reduced in all the four types of tissues ($P < 0.01$). The reduction of LDH activities for liver (6.7 ± 1.05) IU/gm wet tissue), spleen (5.52 ± 1.23 IU/gm wet tissue), kidney (6.36 ± 1.16 IU/gm wet tissue) and bone marrow (6.88 ± 0.66 IU/gm wet tissue) clearly indicates the anticancer effect of BRH_2. But in the experimental group of mice on 35^{th} day after chemotherapy, the level of LDH showed lower value than the normal control.

LDH has received wide attention in the study of neoplasm due to the appearance of 5 interpretable iso-enzymes (Cahn et al., 1962; Markert, 1963) and they are the tetramers of two molecular species, which are present in various tissues in characteristic proportions (Kaplan et al., 1961; Vesell, 1961; and Wroblewski, 1961).

The isoenzyme pattern observed during this investigation made a revelation of 5 isoenzyme bands with high pixel intensity (Pi) in the malignant tissues, while normal liver, spleen, kidney and bone marrow showed lower pixel intensity than its malignant counterpart. ***(Fig.5.2, 5.3, 5.4 and 5.5)***

Ananthanarayan et al. (1978) observed elevated total LDH activity along with the LDH_1, LDH_2 fraction and suggested that LDH_1 and LDH_2 fraction are probably the first to be elevated in early carcinoma in a variety of malignancy. On the otherhand increased level of LDH_3, LDH_4 and LDH_5 have been found to contain predominantly in malignancy (Richards et al.,

1972). Balinsky and his associates (1984) observed all the 5 isoenzymes in the lung cancer and noted significantly higher proportion of LDH_4 and LDH_5 also reported by Heckl & Fogh (1968), while the LDH_1, LDH_2, and LDH_3 were significantly lower. Wang (1991) detected decrease in LDH_2 and LDH_3 and increase in LDH_4 and LDH_5 in the experimental cervical carcinoma of mice. Polivkova et al., (1988) reported higher percentage of LDH_2, LDH_3 and LDH_4 in the methylcholanthrene introduced blood of rat.

The difference in LDH composition in malignant tissues reflect the difference in metabolic requirements in them. It has been pointed out that the different forms of LDH appeared to operate at relatively low level of pyruvate whereas LDH_5 may be inhibited by low levels of pyruvate. The prominence of the isoenzymes in malignant tissues depends for their energy needs on glycolysis and this confirms the Warburg's theory of carcinogenesis (1956).

Besides the general isoenzymes some other electrophoretically separable peaks could be detected in malignant tissues. Such type of bands was also described by Romero-Saravia et al. (1988) in human LDH. The appearance of such bands other than the normal isoenzyme bands is perhaps required for the high and increased glycolytic activity during malignancy. As it was shown by Giannoulaki et al. (1989) such type of band disappears during chemotherapy, could also be confirmed from the present findings. ***(Fig 5.5)***

Earlier Von Eyben et al. (1992) showed 80% recovery of serum LDH_1 after cisplatin treatment and concluded that LDH_1 may be used as a tumor marker in human or animal with testicular germ cell tumor.

The Pixel intensity for LDH isoenzyme has been demonstrated for the first time in the assessment of chemotherapeutic activity. The LDH and its isoenzyme can be used as marker to evaluate the prognostic activity of BRH_2. The copper based drug BRH_2 can be used as chemotherapeutic agent against DAL implanted mice evident from the isoenzyme activity.

Summary:

1. LDH activity is considered as marker in malignancy. LDH activity was determined in Dalton's lymphoma implanted Liver, Spleen, Kidney and Bone marrow of C_3H/He mice. LDH activity was significantly enhanced in Liver & Kidney of malignant set over their control, but the increase in spleen and bone marrow was not significant.
2. The significantly depleted LDH activity was recorded in BRH_2 treated groups (after 35 days) in all the tissues from the control group.
3. Variation of LDH isoenzymes were noted in control, malignant and BRH_2 treated tissues. The control liver tissue showed LDH_2

(Pi 125), LDH_3 (Pi 140) & LDH_4 (Pi 95) with sharp band, but the DAL implanted liver exhibited four prominent bands LDH_1 (Pi 105), LDH_2 (Pi 125), LDH_3 (Pi 145) and LDH_5 (Pi 95). The BRH_2 treated liver after 20 days showed a distinct band i.e. LDH_3 (Pi 120) and in 35 days demonstrated prominent bands of LDH_1 (Pi 100), LDH_2 (Pi 100) and LDH_3 (Pi 90).

4. Control spleen exhibited four prominent bands of LDH_1 (Pi 125), LDH_2 (Pi 160), LDH_3 (Pi 125) and LDH_4 (Pi 190). The DAL implanted spleen showed identical prominent bands of LDH_1 (Pi 155), LDH_2 (Pi 175), LDH_3 (Pi 150) and LDH_4(Pi 130). The BRH_2 treated spleen after 20 days suggested LDH_4 (Pi 140) and LDH_5 (Pi 145) and after 35 days LDH_1 (Pi 150), LDH_3 (Pi 125) and LDH_4 (Pi 140) bands.

5. The control kidney exhibited two prominent bands of LDH_2 (Pi 90) and LDH_3 (Pi 95); but the lymphoma implanted kidney showed distinct band of LDH_1 (Pi 110), LDH_2 (Pi 145) and LDH_3 (Pi 150). In the BRH_2 treated kidney after 20 days the LDH_2 (Pi 140), LDH_3 (Pi 110) and LDH_4 (Pi 100) were prominent while on 35^{th} day one such prominent band of LDH_4 (Pi 105) was noted.

6. Control bone marrow exhibited LDH_1 (Pi 120), LDH_2 (Pi 170) and LDH_3 (Pi 125) as sharp bands but the DAL implanted tissue showed LDH_1 (Pi 140), LDH_2 (Pi 175) and LDH_3 (Pi 125) as prominent bands. BRH_2 treated bone marrow after 20 days showed LDH_1 (Pi 110), LDH_2 (Pi 170) and LDH_5 (Pi 150) and after

35^{th} days shows LDH_1 (Pi 90) and LDH_3 (Pi 140) prominent bands.