

## DETERMINATION OF LIPID PEROXIDATION IN LIVER KIDNEY AND HEART OF WHITE MALE ALBINO RATS

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### ABSTRACT

The experiment has been carried out on Albino Rats treated with Doxorubicin and Etoposide. The treated Rats showed significant weight reduction as compared to the control Rats. The protein content in hepatic, renal and cardiac tissues of treated Rats were found different than the control. The Concentration of malondialdehyde (MDA), as an indicator of Lipid peroxidation, was measured in the liver, kidney and heart by the method of Hermann Esterbauer and Kevin H. Cheeseman. The finding of the research shows that these drugs are effective for treatment of the Carcinogenic effects that could be occurred in mammalian species.

**Key words:** Lipid peroxidation, liver, kidney, heart, white male albino rats.

### INTRODUCTION

Lipid peroxidation in tissue and tissue fraction represents a degradative process, which is the consequence of the production and the propagation of free radical reactions primarily involving membrane polyunsaturated fatty acids and has been implicated in the pathogenesis of numerous diseases including atherosclerosis, diabetes, cancer, and rheumatoid arthritis as well as in drug associated toxicity, postischemic reoxygenation injury and aging<sup>1</sup>.

Doxorubicin belongs to the class of anthracycline. The anthracycline antibiotics are cytotoxic compounds that interact with mammalian cells to inhibit cell growth and viability at low concentration<sup>2,3</sup>. Doxorubicin (Dox), a 14-hydroxy derivative of daunorubicin (DAU), followed in Dimarco *et al.*<sup>4</sup>. Doxorubicin (Dox) was introduced in cancer chemotherapy because of an improved therapeutic effect in solid tumors when compared to daunorubicin (DAU). Adriamycin is the common name for the Doxorubicin hydrochloride; it is known that adrimycin (ADR) inhibits RNA and DNA synthesis through interaction with DNA double helix, interfering in cell division<sup>5</sup>.

Etoposide (VP-16) showed encouraging activity in rodent tumors: the life spans of mice given implants of sarcomas and leukemia's were markedly increased. Etoposide was one of the most active plant products ever tested against a murine leukemia (L1210); it was curative for low tumor burdens. The drug was clearly more effective when administered frequently rather than a single dose<sup>6</sup>. The efficacy of Etoposide in clinical applications quickly became apparent in such diseases as small cell lung carcinoma, testicular cancer, lymphomas, leukemia's, brain tumors and Kaposi's. Sarcoma<sup>7</sup>.

### MATERIALS AND METHODS

**Animals:** Adult male Albino Rat's (Haffkines Research Institute, Mumbai) body weight was found to be 240-250 grams. Rats were allowed ad libitum to tap water and standard diet (Amrut laboratory Animal Feed, Mumbai). Rats were divided into three groups as (I) Control, (II) Etoposide (VP-16) (III) Doxorubicin (Dox) treated, were kept three housed per cages in standard polypropylene cages with metal grill on the top. Rats were maintained under a regulated photoperiod (12-hrs light and 12-hrs dark). The temperature was maintained constant at (26 – 28 °C).

### Experimental Design

Doxorubicin (Dox), anticancer drug was interoperitonally administrated into experimental Rats groups no. (III), at the doses of 1 mg / kg thrice a week for 18 doses Ward JA *et al*<sup>8</sup>. The control group received physiological saline 0.5ml/day, which is accounted 52 days (I). The anticancer drug Etoposide (VP-16) was interoperitonally injected into the experimental Rats groups no. (II) at the dose of 1mg / kg body weight and the same period of control.

### Analytical procedures

#### 1) Extraction of Tissue

After treatment the animals were kept at the same laboratory condition for 2-3 days. Animals were anesthetized with ether anesthesia before dissection. After sacrificing the Rats, the organs like liver, kidney and heart were removed and washed with chilled normal saline and used for homogenization. 10% homogenate were prepared by taking 0.5gm of tissue in 5ml of (0.1M sodium phosphate buffer pH 8.0). The homogenate was centrifuged at 9000 rpm for 20 minutes and the supernatant fraction obtained was used for the estimation of antioxidant enzymes. 25% and 5% TCA treated samples were used for estimation of reduced glutathione and lipid peroxidation.

#### II) Malondialdehyde (MDA) determination

The concentration of MDA in the 10% homogenates of liver kidney & heart (prepared in 0.1M sodium phosphate buffer pH 8.0) was determined by thiobarbituric acid (TBA) assay. This is one of the most common method for Lipid peroxidation studies. It is estimated by the method of Hermann Esterbauer and Kevin.H.chee<sup>9</sup>.

#### III) Total protein determination

The concentration of total protein in 10% homogenates (prepared in 0.1M sodium phosphate buffer pH 8.0) was estimated according to Lowery *et al*<sup>10</sup>.

### Statistical analysis

The samples of the treated and control Rats were measured spectrophotometrically at variable wavelengths using double beam Spectrophotometer (Shimadzu wave visible 160A model). The significance of the difference between

the means was calculated by Students "t" test & data expressed as Mean  $\pm$  SEM. Significance was obtained when ( $P < 0.5$ )

The results obtained in this study are reported in Figs 1-10. During the experimental period, the animals treated with Doxorubicin (Dox) and Etoposide (VP-16) treated groups were monitored. At the time of sacrificed all the three groups showed a considerable change in body weight. The data given in (Fig.1) illustrate that body weight of the control group of Rats was found to be uniformly increased. The Doxorubicin and Etoposide treated Rats showed a significant reduction weight when compared to the control group. In consideration with organ weights, the liver weight of Doxorubicin and Etoposide treated groups showed a non- significantly increase comparison with control set of Rat (Fig.2). Kidney weight of Doxorubicin and Etoposide treated groups were found to be non-significantly decreasing as compared to the control group. (Fig.3) Mean heart weight of Doxorubicin and Etoposide treated groups a non- significant decrease as compared to control group. This is shown in. (Fig4).

The refine given in (Fig.5) demonstrates that the present study also assessed the protein level of liver, kidney and heart after treatment. As a result, the total protein content in liver fraction showed significant increases in case of the Doxorubicin treated group but in the Etoposide treated group a non-significant increase. While the total protein content in kidney fractions, in both Doxorubicin and Etoposide treated groups showed significant increase (Fig. 6). Finally total protein content in heart fraction, in Doxorubicin treated group showed non-significant decrease, but in case of Etoposide treated group showed significant decrease (Fig.7).

The concentration of MDA as an indicator of lipid peroxidation was determined in three organs. An increased concentration of end products of Lipid peroxidation is the evidence most frequently quoted for the involvement of free radicals in human diseases. Several studies support hypothesis that Lipid peroxidation products ingested with food or produced endogenously represent a health risk<sup>11</sup>.

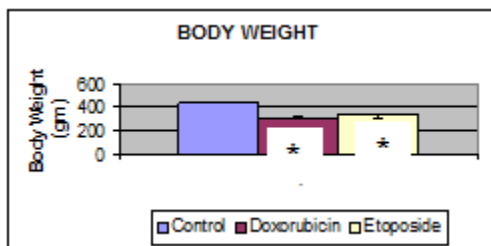


Fig. - 1: Comparison of Body weight in the Control, Doxorubicin and Etoposide in treated male Rats. Each value is the average of six determinations (Mean + SEM). \* Represent significantly decrease from control animal of Rat.

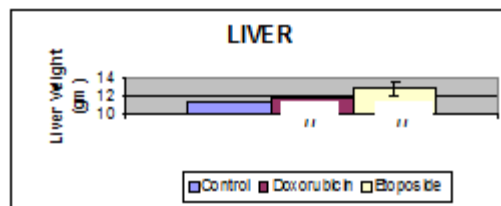


Fig. - 2: Comparison of Liver weight in Control, Doxorubicin and Etoposide in treated male Rats. And values are represented as (Mean + SEM).(n=6). # Represent non-significantly increase from control set of Rat.

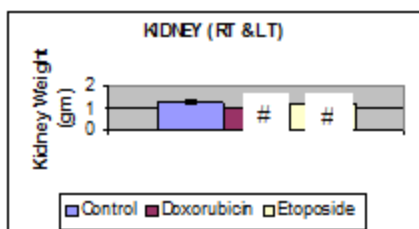


Fig. - 3: Comparison of Kidney weight in Control, Doxorubicin and Etoposide treated male Rats. Values are present as Mean + SEM (n=6). # Represent non-significantly decrease from control animal of Rat.

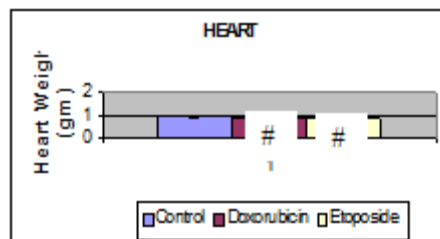


Fig. - 4: Comparison of Heart weight in Control, Doxorubicin and Etoposide treated male Rats. Values are expressed as Mean + SEM (n=6). # Represents non-significantly decrease from control counter part.

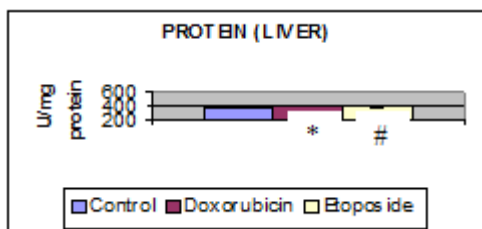


Fig. - 5: Comparison of total protein (Liver) in Control, Doxorubicin and Etoposide treated male Rats. Each value is the average of six determinations (Mean + SEM). \* Represent significantly increase in Doxorubicin while). # Represent non-significantly increases in Etoposide from control values (P < 0.5).

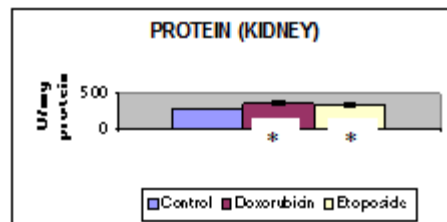


Fig. - 6: Comparison of total protein (Kidney) in Control, Doxorubicin and Etoposide treated male Rats. Each value is the average of six determinations (Mean + SEM). # Represent non-significant decrease while \* Represent significantly increase in both cases from control values (P < 0.5).

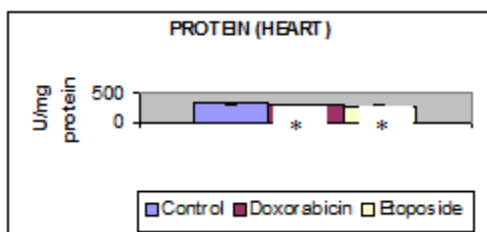


Fig. 7: Comparison of total protein (Heart) in Control, Doxorubicin and Etoposide treated male Rats. Each value is the average of six determinations (Mean + SEM). \* Represent significantly increase in both cases from control values (P < 0.5).

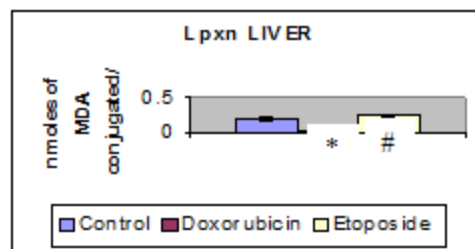


Fig. 8: Comparison of total LPXn content in liver of Control, Doxorubicin and Etoposide treated male Rats. Values are presented as Mean + SEM.\* Represents significant decrease in Doxorubicin while # Represent non-significant change in Etoposide as compared to the saline injected Rats.

Total MDA (Hepatic) formed in the Doxorubicin treated group showed a significant decrease but in the Etoposide treated group did not show any significant change as compared to

the control group as shown in (Fig .8). Where as total MDA (Renal) content in the Doxorubicin treated group showed a significant decrease while in the Etoposide treated group a non-significant change

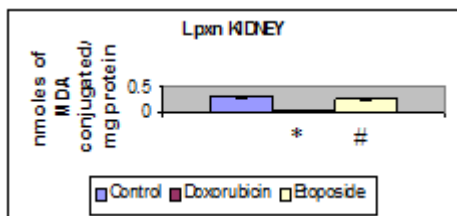


Fig. - 9: Comparison of total LPXn content in kidney of Control, Doxorubicin and Etoposide treated male Rats. Values are presented as Mean + SEM. \* Represents significant decrease in Doxorubicin while # Represent non-significant change in Etoposide as compared to the saline injected Rats.

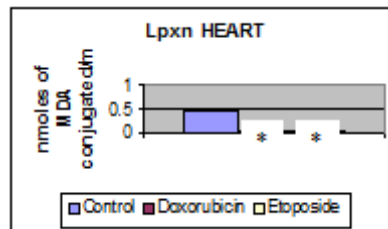


Fig. - 10: Comparison of total LPXn content in heart of Control, Doxorubicin and Etoposide treated male Rats. Values are presented as Mean + SEM. \* Represents significant change in both cases as compared to counter part.

was observed as compared to the control counter part. The total MDA (Cardiac) formed in the tissue of the Doxorubicin and Etoposide treated groups showed a significant decrease as compared to the saline injected Rats. Lipid peroxidation is a major cardiotoxic indicator of Doxorubicin, particularly under the conditions of acute exposure. It has been found that Doxorubicin more selectively induces this oxidative damage to the heart relative to other organs such as the liver in mice<sup>12</sup>. The present study was undertaken to assess the oxidative status of hepatic, renal and cardiac tissues in the treated

groups Doxorubicin, Etoposide & Saline. The oxidative stress induced damage as result of peroxidation of polyunsaturated fatty acids (PUFA) called Lipid peroxidation. In fact aldehydes, the end products of Lipid peroxidation are held responsible for the effects Lipid peroxidation distant from their origin.

In Lipid peroxidation, Malondialdehyde (MDA) is an indicator in the organs. The detailed mechanism of action, which leads to malfunctioning of these organs, probably remains unclear.

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