EXPERIMENTAL ANIMALS

The experiments were performed on 12 healthy Swiss albino rats of both sexes weighing 100 to 200 gms obtained from Animal House at Maharani Laxmi Bai Medical College, Jhansi. The animals were maintained on a standard balanced diet.

The animals were divided into 3 groups of 12 animals each; 6 untreated animals served as healthy control.

ADMINISTRATION OF DRUG

Doxorubicin hydrochloride (trade name Doxorubicin Meiji) manufactured by Dong-A Pharma Co. Ltd., Seoul, KOREA marketed in India by Biochem Pharmaceutical Industries, Bombay, was used; 10 mg vial of Doxorubicin hydrochloride was reconstituted with 5 ml sterile normal saline. The reconstituted solution is stable for 24 hours at room temperature and 48 hours under refrigeration (2°C - 8°C). Any unused solution was discarded.

Thirty six rats divided into three groups A, B and C of 12 animals each received the drug intraperitoneally (i.p.) as per following schedule: -
Animals of Group 'A' were given a single intraperitoneal injection of 10 mg/kg doxorubicin, whereas those of Group 'B' received i.p.i. of doxorubicin in doses of 5 mg/kg on two consecutive days and the animals of Group 'C' were administered with doxorubicin in doses of 1 mg/kg on ten consecutive days.

Sacrifice of Animals:

After the last injection the animals were sacrificed in batches on day 1, 3, 7 and 14 under ether anesthesia. At autopsy the heart along with covering pericardium was removed carefully and washed thoroughly in cold isotonic normal saline. Its gross features were recorded. The hearts were preserved in 10% formal saline for detailed histopathological examination.

HISTOLOGY OF HEART

From each heart four serial transverse parallel blocks including one at the apex, another from ventricles, the third from atrioventricular junction and the last from base of the heart, so as to include large blood vessels, were obtained. From each block 3-5 serial paraffin sections 3-4 µm thick were obtained and stained with Harris's Haematoxylin and Eosin; V.G., Reticulin and PAS stains were also used whenever needed.
The sections were examined in detail using a light microscope and the findings of the histological examination in terms of cytoplasmic vacuolization, myofibre necrosis, inflammatory and mesenchymal reactions and interstitial haemorrhage were recorded and arbitrarily graded using half point scale. The distribution and nature of these lesions was also recorded. The severity of the lesions was graded as 'Mild' (+), 'Moderate' (++) or 'Marked' (+++) as described below:

The individual sections were graded separately and the average findings were expressed in results. Associated findings e.g., periocarditis, valvulitis, lymphatic involvement and vascular changes were also noted. The observations were recorded using half point scale as detailed below. The changes were graded on a scale of 0 to 1.5 corresponding to absent, mild, moderate and marked.

**CYTOPLASMIC VACUOLIZATION**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No vacuolization seen</td>
</tr>
<tr>
<td>0.5</td>
<td>Isolated minimal foci of characteristically vacuolated myocytes.</td>
</tr>
<tr>
<td>1</td>
<td>Larger and more numerous foci of vacuolated myocytes.</td>
</tr>
<tr>
<td>1.5</td>
<td>Generalized vacuolization of myocytes covering more than 75% of the high power field.</td>
</tr>
</tbody>
</table>
MYOFIBRE NECROSIS :

Mild (+) : Scattered small foci consisting of 1 to 2 necrosed myofibres.

Moderate (++): Scattered foci consisting of 3 to 5 necrosed myofibres.

Marked (+++): Confluent foci of myofibre necrosis or when scattered, larger foci consisting of more than 5 necrosed myofibres.

INFLAMMATION

Mild (+) : Focal collection of less than 10 inflammatory cells.

Moderate (++): Focal collection of 10-20 inflammatory cells.

Marked (+++): Diffuse infiltration with inflammatory cells in a wide area or focal collection of more than 20 cells.

HAEMORRHAGE :

The interstitial haemorrhages, which were few or multiple, depending upon their size were arbitrarily graded as small or large.
INJURY SCORE

In order to have some quantitative assessment of myocardial injury in different rats, the attempt was made for numerical scoring of the injury. For this purpose the points awarded to a lesion on half point scale were added and their sum total represented the injury score for a particular section. Average score of all the sections represented the injury score of that particular heart, and the mean injury score of a particular batch and/or a group of animals, thus could be calculated and used in further assessment and comparison of intensity and severity of myocardial lesions in various groups of these animals.