MATERIAL AND METHODS
The present study was carried out in the Department of Biochemistry, Medical College, Rohtak, using albino rats as experimental animals. Albino rats of both sexes weighing 125-250 gms were taken from the Departmental Animal Room for the study. It was ensured that the experimental animals were disease free. They were fed on 'Gold Mohur Rat Feed' (Liptons India Ltd.) Food was available to the animals ad-libitum and the animals had free access to water. They were divided into two groups and caged separately.

**Method of Celphos Poison:**

One Tablet of Celphos weighing 3 gms was thoroughly and uniformly suspended in 25 ml of dehydrated oil (the dehydrated oil was prepared from ordinary refined oil (Ginni) by keeping it in hot-air oven at 110°C for 12 hours. This uniform suspension served as a stock solution of celphos. This solution was prepared regularly after every 10 days or as and when it was required for the experiment. The dilute solution of Celphos was prepared by diluting stock suspension in the ratio of 1:10. This solution was prepared afresh each time. This dilute solution of Celphos poison was used for poisoning the rats.
Feeding of poison suspension solution

3 ml of dilute Celphos poison suspension was administered through an infant feeding tube to each of the animals of the poisoned group. Similarly, the rats of control group were given 3 ml of dehydrated oil.

The blood was withdrawn 15-20 min. after the oral administration of Celphos suspension or dehydrated oil from two groups of animals into the bottles with the help of heparin-rinsed syringes, directly from heart. Moreover, after withdrawing blood, the organs e.g. brain, lung and liver were also removed from nine animals of the two groups.

Processing of Red Blood Cells

The blood was processed according to the method described by Stock et al, 1972 and Snyder et al, 1992, was centrifuged and the supernatant plasma aspirated. Then, equal volumes fortified buffer saline (FBS) was added for washing each time, for three times. After washings, red cells were suspended in 5 ml of FBS and mixed properly. The haemoglobin concentration was estimated by cyanmethaemoglobin.
method using Drabkins situation (Drabkins et al., 1973). The haemoglobin concentration of the cell suspension was adjusted to 5 g%. This diluted cell suspension was taken for estimation of malondialdehyde (Snyder et al., 1991).

**In Vitro Exposure of RBC suspension to Phosphine:**

Phosphine gas was liberated by the interaction of aluminium phosphide with water (Hayes, 1992). A tablet of Calphos was placed in a 25 ml conical flask fitted with rubber tubing and a glass pipette. The tap water (10 ml) was added to the conical flask to generate phosphine gas. The evolving gas was bubbled in the RBC suspension from each of the control rat for 5 minutes.

**Estimation of Malondialdehyde (MDA):**

The generation of MDA was estimated by the method of Snyder et al. (1991) and Stock et al. (1972).

**Principle:** Thiobarbituric acid (TBA) is believed to react with malondialdehyde to form a stable pink with an absorption maximum at 535 nm. The simplicity and sensitivity of the TBA method had made it most widely used indicator of lipid peroxidation.
Resents

1. 0.2M-KH$_2$PO$_4$: 6.8 gms of KH$_2$PO$_4$ was dissolved in 100 ml of distilled water.

2. 0.2M-Na$_2$HPO$_4$: 8.9 gms of Na$_2$PO$_4$·2H$_2$O was dissolved in 100 ml of distilled water.

3. 0.15M-NaCl: 3.7 gms of sodium chloride was taken and dissolved in one litre of distilled water.

4. Phosphate buffer saline (PBS): 17.6 ml of 0.5M KH$_2$PO$_4$ and 60.8 ml of 0.5M Na$_2$HPO$_4$ solutions were mixed and then made up to one litre with distilled water. 100 ml of above solution was taken and its pH was adjusted to 7.45. One litre of 0.15M sodium chloride solution was added to the above buffer solution.

5. Fortified buffer saline with 2.0mM sodium azide and 4.0mM glucose (PBS·N): One litre of buffered saline added 136.5 mg of sodium azide and 738 mg glucose. This is mixed uniformly and stored at 4°C. If any suspended particles appear it is discarded and freshly prepared.

6. TC·Arsenite solution: 140 gm of Trichloroacetic acid (TCA) was added to 250 ml of distilled water and it was followed by the addition of 6.3 mg of sodium arsenite. The mixture was dissolved by heating. After cooling the above solution, the volume was
made up to 500 ml with distilled water. It was filtered with glass wool.

7. Thiobarbituric acid (TBA): 500 mg of TBA was dissolved by heating in 20 ml of distilled water and 2.5 ml of 1M-sodium hydroxide. It was cooled and filtered. The volume of this solution was made to 50 ml with distilled water. This solution was changed asc and when there are visible precipitates.

8. Hydrogen peroxide: It was prepared according to the method described by Beutler (1975). The optical density of 0.9 ml of 1:10 dilution of phosphate buffer, pH 7.0 was measured at 230 nm (O.D.). 0.1 ml of 1:10 dilution of H₂O₂ (30%) solution was added to the above mentioned phosphate buffer solution and again the optical density of the resulting solution was recorded (O.D.). Since the molar extinction coefficient of H₂O₂ at 230 nm is 0.071 (Chance, 1954). The H₂O₂ concentration (c) of the 1:100 dilution of peroxide solution is

\[ 141 \times (c_{\text{dilute}} \times 0.01) \text{ mM} \]

To dilute to 6.0 mM required for the assay, 1 ml of 1:100 dilution of H₂O₂ was made to C/6.0ml with distilled water.
9) Drabkin's solution (Dacie and Lewis, 1975):

Drabkin's solution was prepared by adding 200 mg potassium ferricyanide, 50 mg potassium cyanide and 140 mg potassium dihydrogen phosphate in one litre of deionised water. The clear solution was stored in amber coloured bottles at room temperature. The reagent was discarded when turbidity appeared.

Estimation of Haemoglobin Concentration:

To 4 ml of Drabkin's solution in a stoppered tube, 0.02 ml of red cell suspension was added. After 10 minutes absorption was read against a reagent blank on a Erma Haemoglobinometer at 540 nm.

Procedure (Malonyl-diadehyde estimation):

Red cell suspension (2.0 ml) prepared as described earlier, from control and poisoned rats was taken in the test tube and equilibrated at 37°C in shaking water bath for 10 minutes. Then, 2 ml of 6M H₂O₂ solution was added from the side walls. The time noted as (zero time). This mixture was incubated for 2 hours at 37°C.

3 ml of incubated red cell suspension with buffered saline and with H₂O₂, was added to 2 ml
TCA -Arsenite solution. The mixture was kept for 15 minutes. It was centrifuged at 4000 rpm at 10 min.
3 ml of supernatant was transferred to a 15 ml centrifuge tube, and to this 1,0 ml of TBA solution was added. An air condenser was fitted to the tube and mixture incubated for exactly 15 minutes in boiling water bath.
The tubes were then cooled.

The absorption of the mixture was recorded at 532 nm and 600 nm with Bosch and Lomb Spectronic-20 setting the instrument zero with distilled H2O.

Calculations:

\[ \text{MDA} = \text{OD}_{532} - \text{OD}_{600} \times 900 \text{ nmole/gm Hb} \]

Tissue MDA assay

The estimation was done according to the method as described by Ohkawa et al (1979).

with heat induced reaction: One mole of MDA (a secondary lipid peroxidation product) reacts with 2 mole of TBA in the acid solution and forms trimethine pink coloured substance.

As mentioned in materials and methods, organs from rats were removed and were perfused with ice cold normal saline (0.9% NaCl). Then, 1 gm of tissue
was weighed and to it 9.0 ml of 1.15% KCl solution was added. The mixture was homogenized using a teflon homogenizer. This homogenate were subsequently used for MDA estimations.

**Reagents:**

1. 8.3% KCl: 4.05 gm of KCl (DDH) was dissolved in 50 ml of distilled water.
2. 20% Acetic acid: 1.0 ml of glacial acetic acid was diluted to 5 ml with distilled water. The pH of this solution was adjusted to 3.5 with NaOH. It was prepared afresh just before use.
3. 1.15% HCl: 1.15 gm of HCl was dissolved in 100 ml of distilled water.
4. 0.04 TBA aqueous solutions: 200 mg of TBA was dissolved in 25.0 ml distilled water after sufficient warming. The solution was changed if any precipitates or suspended particles appear.
5. N-Butanol: Pyridine (15:1 V/V): This was prepared freshly by adding 90 ml a-butanol and 6.0 ml of pyridine.

**Procedure:** 0.1 ml of tissue homogenates were taken in 20 ml test tubes and to this 0.2 ml of 8.1% sodium dodecyl sulphate (SDS) was added. It was followed by the addition of 1.5 ml of 20% Acetic acid (pH 3.5).
1.5 ml of 0.86 aqueous TBA and 0.7 ml of distilled H_2O. The test tubes were placed in a oil bath of 95°C for 60 minutes after properly sealing them with aluminium foil. The tubes were then taken out and cooled under running tap water. To each of the tube, 1 ml distilled water and 5.0 ml of n-butanol pyridine (15:1 V/V) mixture was added. It was shaken vigorously then the tubes were centrifuged at 4000 rpm for 10 minutes. Absorbance of carefully aspirated organic layer was measured at 532. The tests were run in duplicate on each sample.

**Calculations:**

Tissue MDA = \(15.860_{332}\) n.moles/0.1 ml of Homogenate.

The levels of MDA in tissues have been expressed in n.moles/0.1 ml homogenate as have been done by Place et al (1966).

**ESTIMATION OF TRANSAMINASES (COOT, SOPT)**

This was done as described by Reitman and Frankel (1957).

**Principle:** Aspartate transaminase effects the conversion of L-keto glutarate and aspartate to glutamic and oxaloacetate respectively by amino group transfer.
The exaloeate spontaneously decarboxylates to pyruvic acid, which couples with 2,4-dinitrophenyl hydrazine to produce a coloured complex whose absorbance in alkaline solution measured at 505 nm.

Alanine transaminase effects the conversion of L-α-ketoglutarate and α-alanine to glutamic and pyruvate respectively by amino group transfer. The pyruvic acid thus formed is coupled with 2,4 dinitrophenyl hydrazine to produce a coloured complex whose absorbance in alkaline solution is measured at 505 nm.

Reagents:

Phosphate buffer (pH 7.4): 5.65 gms of disodium hydrogen phosphate and 1.35 gms of potassium dihydrogen phosphate were dissolved in 500 ml of distilled water. The pH was adjusted to 7.4, if required.

2) ECOT substrates: 6.65 gms of aspartic acid was dissolved in 43 ml of 1N-NaOH. Then, 73 mg of L-α-ketoglutaric acid to above solution was added and the volume was made upto 250 ml with phosphate buffer.

3) EGPT substrates: 4.5 gms of alanine was dissolved in 43 ml of distilled water and to this 1.2 ml of 1N-NaOH was added. Then, 73 mg of L-α-ketoglutaric acid was added to it and the volume was made to 250 ml with phosphate buffer.

4) Dinitrophenyl hydrazine (DNPH): 100 mg of DNPH
was dissolved in 50 ml of concentrated hydrochloric acid and the volume made up to 50 ml with distilled water.

3) Pyruvate standard: 44 mg of Pyruvic acid was dissolved in 100 ml of distilled water.

Procedure:

The substrate was incubated for 3 min. at 37°C and processed as follows:

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>Blank</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Serum</td>
<td>0.1 ml</td>
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<td>0.1 ml</td>
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</tbody>
</table>

The tubes were incubated at 37°C for half an hour for SGPT and one hour for SGOT.

After incubation time was completed, 0.5 ml of DNPH solution was added to all the tubes. After 20 min., 0.1 ml of serum was added to the control tube.

This was followed by the addition of 5 ml of NaOH (0.4 N) to each of the test tube. After 10 min., the optical density of the solutions from all the test tubes was recorded at 505 nm by setting the instrument to 100% transmission with water.

Calculations:

\[
\text{SGOT} = \frac{O_{D_{57}} - O_{D_{59}}}{O_{D_{59}}} \times 67 \text{ u mol pyruvate/min/L}
\]
The calculated pyruvate was converted to international units (I.U.) from standard tables.

\[ \text{SGPT} = {O_a D_c T_o - O_a D_c C_o} \times 135 \text{ u mol Pyruvate/min/L} \]
\[ O_a D_c E_o = O_a D_c B_e \]