The protective influence of *Moringa oleifera* against mercury and radiation induced changes in the kidney of Swiss albino mice have been studied in the present investigation.

**EXPERIMENTAL ANIMALS**

The adult healthy male Swiss albino mice (6-8 weeks old) were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar. The Govt. Dungar College, Bikaner is registered under CPCSEA, New Delhi (registration no. 1066/GO/Re/S/07/CPSEA) and has its own Institutional Animal Ethics Committee (IAEC). In view of the above, the present experiments were conducted under the supervision of IAEC of the College. The animals were housed in polypropylene cages and maintained on balanced mice feed and tap water *ad libitum*. They were acclimatized to laboratory conditions before use. Occasionally tetracycline water was provided as a precaution against infection. The temperature of the room was maintained between 22-27ºC.

**MERCURIC CHLORIDE**

The mercury salt in the form of mercuric chloride of analytical grade was used for the present study. It was purchased from Ranbaxy Laboratories Ltd., India. It was administered orally in drinking water at the dose of 0.5ppm.
SOURCE OF RADIATION

The animals were exposed by the Cobalt-60 gamma radiotherapy source (Theratron) of AECL make, obtained from Canada. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan). The animals were irradiated at the dose rate ranging from 0.95 Gy/min to 1.97 Gy/min. The dose was calculated at the mid point by multiplying dose rate and tissue air ratio. The tissues of Swiss albino mice were assumed to be equivalent to human soft tissues.

MORINGA OLEIFERA

The aqueous extract of Moringa oleifera was procured from the Umalaxmi organics private limited, Jodhpur (Raj). The plant extract of Moringa was fed orally at the dose of 150 mg/kg body weight. The Moringa extract was given daily from seven days prior to individual or combined treatment of mercuric chloride and radiation and continued up to the last autopsy interval.

DESIGN OF EXPERIMENTATION

In order to investigate the protective effect of Moringa oleifera against radiation and Mercuric induced changes in mice kidney the animals were divided into following groups:

Group – I (Sham-irradiated animals) The animals of this group were sham- irradiated and served as control (normal) group.
Group - II  (Mercuric chloride treated animals)

All the animals of this group were orally fed with mercuric chloride solution at the dose rate of 0.5 ppm *ad libitum* in drinking water continuously till the end of experiment.

Group - III  (Irradiated animals)

The animals of this group were exposed to sub-lethal doses of gamma radiation from Cobalt-60 source. This group was further divided into two sub-groups on the basis of radiation dose received:

Sub-group III a: 2.5 Gy
Sub-group III b: 5.0 Gy

Group - IV  (Animals treated with radiation and Mercuric chloride)

All the animals of this group were orally fed with Mercuric chloride solution (0.5 ppm) and also exposed to different doses of gamma radiation. This group was further divided into two sub-groups on the basis of radiation dose received:

Sub-group IV a: 2.5 Gy + Mercuric chloride
Sub-group IV b: 5.0 Gy + Mercuric chloride

Group - V  (Mercuric chloride and Drug treated animals)

The animals of this group were orally fed with Mercuric chloride (0.5 ppm) and also received *Moringa oleifera* orally for seven days at a dose of 150 mg/kg body
wt./animal/ day prior to Mercuric chloride treatment and continued up to the last autopsy interval.

Group - VI (Radiation and drug treated animals)

The animals of this group were exposed to gamma radiation from Co$^{60}$ source. The *Moringa oleifera* was given seven days prior to irradiation and continued up to last autopsy interval. This group was further divided into two sub-groups on the basis of radiation dose received:

Sub-group VI a: 2.5 Gy + *Moringa oleifera*
Sub-group VI b: 5.0 Gy + *Moringa oleifera*

Group - VII (Radiation, Mercuric chloride and drug treated animals)

The animals of this group were orally fed with Mercuric chloride solution at the dose rate of 0.5 ppm and received *Moringa oleifera* orally (150 mg/kg/b.wt./animal/day) for seven days prior to irradiation and Mercuric chloride till the last autopsy day of experiment. This group was further divided into two sub-groups on the basis of radiation dose received:

Sub-group VII a: 2.5 Gy + Mercuric chloride + *Moringa oleifera*
Sub-group VII b: 5.0 Gy + Mercuric chloride + *Moringa oleifera*

**Autopsy**

A minimum of five animals from groups II to VII were sacrificed by cervical dislocation and autopsied at each post-treatment intervals of
1, 2, 4, 7, 14 and 28 days. Five sham-irradiated mice were also sacrificed in the similar manner.

The following studies were taken into consideration for the present study

(I) **Reno-somatic index** - The weight of kidney was recorded and expressed as reno-somatic index (gm/100 gm body wt.)

\[
\text{Reno-somatic index} = \frac{\text{Weight of kidney}}{\text{Weight of body}} \times \frac{100}{1}
\]

(II) **Histological studies** - After sacrificing the animals, pieces of the kidney were fixed in Bouin’s fixative for 24 hours. The tissues were washed in water to remove excessive of fixative, dehydrated in graded series of alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5 \(\mu\)m and stained in Harris haematoxyline and alcoholic eosin.

(III) **Biochemical studies** - Rest of the kidney was kept at \(-20^0\)C for the estimation of following biochemical parameters:

1. Total proteins [Lowry *et al.*, 1951]
2. Glycogen [Montogomery, 1957]
4. Acid phosphatase activity [Fiske and Subbarow, 1925]
5. Alkaline phosphatase activity [Fiske and Subbarow, 1925]
6. DNA [Certiotti, 1952]
7. RNA [Ceriotti, 1955]
1. Total Proteins:

The quantitative estimation of total proteins was carried out by the method of Lowry et al. (1951).

**Principle:**

Protein reacts with the folin-ciocalteau reagent to give a coloured complex. The colour so formed is due to the reaction of the alkaline copper with the protein as in the biuret test and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of colour depends on the amount of proteins present.

**Calculations:**

The content of total proteins (mg/gm of tissue wt.) was calculated by the following formula:

\[
\text{Total proteins content (mg/gm tissue weight)} = \frac{\text{O.D. of test}}{\text{O.D. of standard}} \times \frac{\text{Conc.of standard tissue taken}}{0.5} \times 4 \times 1000
\]

2. Glycogen:

The glycogen was estimated quantitatively by the method of Montogomery (1957).

**Principle:**

The Procedure is based on the phenolic sulphuric acid method for the determination of alkali soluble polysaccharides in tissues. Carbohydrates treated in the presence of sulphuric acid and phenols
undergo dehydration with the formation of hydroxy aldehydes, which are subsequently converted to furfural derivatives. The reaction gives rise to a pink colour, the intensity of which is proportional to the amount of glycogen present.

**Calculations:**

The glycogen content (mg/gm of tissue wt) calculated by the following formula:

\[
\text{Glycogen content} = \frac{\text{O.D. of test}}{\text{O.D. of standard}} \times \frac{\text{Conc. of standard}}{\text{tissue taken}} \times 4 \times \frac{1000}{1}
\]

(mg/gm of tissue wt)

3. **Cholesterol**

The cholesterol was estimated by the method of Oser (1965).

**Principle**

The procedure is based on extraction and oxidation of cholesterol present in tissue by an acidic solution of ferric chloride and subsequent addition of sulphuric acid. This reaction gives rise to a brown coloured complex, which has been used as the base of analysis of total cholesterol.

**Calculations**

The content of cholesterol (mg/gm of tissue weight) was calculated by using the following formula

\[
\text{Cholesterol content} = \frac{\text{O.D. of test}}{\text{O.D. of standard}} \times \frac{0.2}{1} \times \frac{1000}{\text{tissue taken}}
\]

(mg/gm tissue wt)
4. Acid Phosphatase Activity

Acid phosphatase activity in the tissue was estimated by the method of Fiske and Subbarow (1925).

**Principle**

The proteins are precipitated with trichloroacetic acid. The proteins free filtrate is treated with an acid molybdate solution which forms phosphomolybdic acid from any phosphate present. The phosphomolybdic acid is reduced by the addition of 1, 2, 4-amino nephtol sulphuric acid reagent, to produce a blue colour whose intensity is proportional to the amount of phosphate present.

**Calculation**

The acid phosphatase activity was calculated by using following formula

\[
\text{The liberated phosphorus in tissue (mg pi/gm/hour) = } \frac{(\text{O.D.of experimental} - \text{O.D.of control}) \times 0.04 \times 3 \times 1000 \times 2}{\text{O.D.of standard} \times \frac{1}{2} \times \text{tissue taken} \times 1}
\]

5. Alkaline Phosphatase Activity

This parameter was also estimated according to the method of Fiske and Subbarow (1925).

The principle, reagents, procedure observations and solutions were similar to those of acid phosphatase except for buffer used, which was different.
6. DNA and 7. RNA

The estimations of DNA and RNA were carried out by Ceriotti (1952) and Ceriotti (1955) method respectively.

Principle

The extraction of DNA and RNA is done using perchloric acid. Final precipitation is done by 0.6 N NaOH. For the development of colour, diphenylamine reagent is added to the precipitate and orcinol is added to the supernatant, which represent the DNA and RNA fractions respectively.

The DNA and RNA content (mg/gm of tissue weight) was calculated as follows

The content of DNA

\[
\text{The content of DNA} = \frac{\text{O.D. of unknown}}{\text{O.D. of standard}} \times \frac{\text{Conc. of Standard tissue taken}}{28} \\
\text{(mg/gm tissue weight)}
\]

The content of RNA

\[
\text{The content of RNA} = \frac{\text{O.D. of unknown}}{\text{O.D. of standard}} \times \frac{\text{Conc. of Standard tissue taken}}{15} \\
\text{(mg/gm tissue weight)}
\]

Statistical Method

After obtaining the mean values of control and experimental groups, the standard error (S.E.) of mean was calculated by the following formula

\[
\text{S.E.} = \sqrt{\frac{n(\sum x^2) - (\sum x)^2}{n^2(n-1)}}
\]
Where,\n\[ n \] - number of readings
\[ \Sigma \] - Sigma (sum of)
\[ x \] - Individual reading

The ‘t’ values were calculated by the method of student ‘t’ test.

\[
t = \frac{m_1 - m_2}{\sqrt{(x_1)^2 + (x_2)^2}}
\]

Where,
\[ m_1 \] and \[ m_2 \] = mean of control and experimental values respectively
\[ x_1 \] and \[ x_2 \] = standard errors of control and experimental values respectively with the help of ‘t’ table the level of significance was noted.

Degree of freedom (d.f.) = \[(n_1 + n_2) - 2\]

Where
\[ n_1 \] - no. of variation in control group
\[ n_2 \] - no. of variation in experimental group

Significance levels are expressed in 'P' value as
\[ \text{<0.05} \] - Significant
\[ \text{<0.01} \] - More Significant
\[ \text{<0.001} \] - Highly Significant
\[ \text{N.S.} \] - Non Significant