MATERIAL & METHODS
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Traditional and folklore medicine bequeathed from generation to generation is rich in domestic recipes and communal practice. Encompassing concepts and methods for the protection and restoration of health, traditional medicine has served as a fount of alternative medicine, new pharmaceuticals, and healthcare products. The best known examples of traditional medicine, differing in concept and protocol, are well-developed systems such as acupuncture and ayurvedic medicine that have been widely used to conserve human health in China and India. Medicinal plants can make an important contribution to the WHO goal to ensure, that all peoples, worldwide, will lead a sustainable socio-economic productive life. A most important prerequisite for promoting the use of new contraceptive is to disseminate, correct and balance information about their risks, benefits and side effects to potential user and provides as well as to other concerned groups. Therefore, the present plant, Abrus precatorius, was selected because it shows spermicidal effects with no adverse effect on liver function.

SELECTION OF PLANT MATERIAL

Though many indigenous plants have shown to prevent birth only a few have so far been investigated for antispermatogenic activity. For the present study, Abrus precatorius seeds known for its medicinal effect was selected.
Plant *Abras precatorius* with seeds
Abrus precatorius is the member of family Leguminous with characteristic red and black seeds. Its leaves are pinnate with many leaflets arranged in pairs. The plant bears orange pink flowers, sometimes yellowish or reddish purple in colors arranged in racemes. The pods are brownish which embody pendulous seeds. The leaves, roots and seeds of Abrus precatorius are used for medicinal purposes, a practice most probably dating back to antiquity (Ivon 2003).

In India it is commonly known as Ratti, Aainud –dik, chunhati, crab’s eye. Crab’sstone, Gaungchi, Gchi, Ghongchi, Ghumachi, Ghun, Guinea pea, Gunch, Gundumani, Gunja, Guri-ginja, Gurivinda, Gurje-tiga, Indian-licorice, jequirity, KalyaniKoonch, Kunni, Latuwani, Olinda, safed chirami, sonkach, rosary pea, precatory bean etc. The seeds of Abrus precatorius are used by tribal people for common ailments related to reproduction. Since this plant has abortificant effect in females, it is used criminally for poisoning and aborting cattle.

Pharmacological activities and clinical trials proved that this plant possesses antifertility, agglutinin, analgesic, anthelmintic, antibacterial and antidiarrhoeal activity, anti-estrogenic effect, antifungal activity, antispermatic effect, antitumor and antiviral activity, CNS depressant activity, contraceptive and interceptive
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effect, diuretic and hypoglycemic activity, HIV inhibition, spermicidal effect and uterine relaxation activity.

A seeds boiled in milk is used orally in Unani and Ayurvedic medicines as an aphrodisiac in males. In females, one seed covered with jaggery is swallowed during menstrual period and is sufficient to prevent conception for one year. In Jamaica, it is used as tonic by boiling leaves and roots in milk. In Nigeria, its roots are used as an antimalarial and anticonvulsant. In Taiwan, decoction of dried root is taken orally to treat bronchitis and hepatitis. In Tanzania decoction of root and leaf sap is taken orally for asthma and is also used as aphrodisiac.

**COLLECTION AND PREPARATION OF PLANT MATERIAL**

The previous work done on this plant has shown that aqueous and methanolic extract of seeds of this plant definitely possesses antispermatogenic properties. Also, it does not cause damage to hepatic tissues (Sharma 2007). Therefore, its effect on renal tissue and blood has been studied in the work.

Fresh specimen of the selected plant was collected by spot collection. The seed of *Abrus precatorius* were procured and dried in shade. After removing dust, plant material was thoroughly crushed in mixer grinder. The powder of the plant material was used for preparing the chloroformic extracts.
**EXTRACTION**

Extraction of the powdered seeds of *Abrus precatorius* was done by soxhlele extractor with thermostatically controlled, heating mantle. The extraction was done with chloroform (LR grade; of Glaxo Laboratories India Limited, Bombay) at 58°C for $12 \times 3$ h. The Extract was dried in hot air oven at 60°C. The dried extract was kept in air tight container, with a small crystal of thymol to prevent fungal growth, in refrigerator.

**Chromatography**

The dried extract was dissolved in different solvents and chromatographed over deactivated silica gel (60-120 mesh) in a glass column. The different fraction, viz., methanolic and aqueous were eluted separately, dried under reduced pressure and used after making required doses in olive oil.

**Experimental Model**

Sexually mature albino rats (*Rattus norvegicus*) of Sprague Dawley strain controlled breed were procured from Jamia Hamdard, Ghaziabad.

Sexually mature male animals usually weighing between 50-150 g were used for the experiments. Before commencement of experiments, the animals were acclimatized in the laboratory for a week on normal diet and water ad libitum. The diet consisted of carbohydrates (69%), proteins
Experimental Animal Albino Rats \textit{(Rattus norvegicus)}
(16%), fats (7%), fiber (6%), mineral salt (2%) and was enriched with vitamins (Cathberton, 1957; Joubert, 1967)

Experimental Design

Experimental animals were randomly only divided into three sets. Each set consisted of two groups of 16 animals each. Out of these two groups of set I, group I served as normal control group, group II served as chloroformic extract of *A. precatorius* seeds of dose 20mg/rat/day.

In Set II, group I served as normal control group, group II served as eluted methanolic fraction of chloroformic extract of *A. precatorius* seeds of dose 2mg/rat/day.

In Set II group I served as normal control group. Group II served as eluted aqueous fraction of chloroformic extract of *A. precatorius* seeds of dose of 2mg/rat/day.

This dose was selected for the present work because in our previous experiments it was observed that this dose successfully cause azoospermia while it does not affect the libido of the animal. Also, this dose did not show any adverse effect of hepatic tissue and liver physiology.

From each group, 8 rats were dissected after 15 days and 8 rats after 30 days of extract feeding during experimentation, all possible efforts were made to keep the animals at a uniform temperature. Necessary precautions
SET I

GROUP I

NORMAL CONTROL

15 DAYS DURATION

30 DAYS DURATION

GROUP II

TREATED

CHLOROFORMIC

20 mg / RAT / DAY

15 DAYS DURATION

30 DAYS DURATION
SET II

GROUP I

NORMAL CONTROL

15 DAYS DURATION

30 DAYS DURATION

GROUP II

TREATED

ELUTED METHANOLIC FRACTION OF CHLOROFORMIC EXTRACT 2 mg / Rat / Day

15 DAYS DURATION

30 DAYS DURATION
SET III

GROUP I

NORMAL CONTROL

15 DAYS DURATION

30 DAYS DURATION

GROUP II

TREATED

ELUTED AQUEOUS FRACTION OF CHLOROFORMIC EXTRACT 2 mg / Rat / Day

15 DAYS DURATION

30 DAYS DURATION
were taken to keep the animals free from any apparent disease as directed by competent authorities from CPCSEA, Chennai.

AUTOPSY SCHEDULE

Twenty four hour after administration of last dose, the animals were weighted and autopsied under local anesthesia and the parameters were assessed at autopsy.

MORPHOLOGICAL STUDIES

For the morphological and biochemical studies the animals were anesthetized with local anesthesia and body weight of each animal was taken on physical balance. The animals were cut open immediately. The morphology of kidney was studied and changes in colour or shape were recorded. The kidney was removed, dried with blotting paper and weighed on chemical balance to the nearest milligram.

HISTOLOGICAL STUDIES

For histological studies, small pieces of kidney were fixed in Bouins-Hollande sublimate. After fixing, the tissues were thoroughly washed in running water and finally in distilled water to remove the excess. Lithium carbonate was used as and when required to remove excess fixative. The tissues were rapidly dehydrated, cleared in sulphur-free xylene and embedded, after making proper orientation, in cerasin rich paraffin wax of 56 to 60 m.p. from BDH.
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The tissue blocks were trimmed. Transverse sections of 6-10 um (micrometer) thickness were cut on "Sartorius rotary microtome" and stained with Iron alum Haematoxylin and eosin.

HAEMATOLOGICAL STUDIES

The animals were sacrificed at the end of experiment. Blood of scarified animals was collected by heart puncture in a dry glass vial with anticoagulant EDTA (ethylene diamine tetra acetic acid). Following parameters were estimated.

(a) **Haemoglobin**: - The amount of haemoglobin was determined by Gowers Haldane's haemometer using 0.1N HCL as diluting. The graduated haemoglobin tube was filled with N/10 Hcl up to mark 8. Now the blood was sucked in the haemoglobin pipette up to mark 20 cu mm. Subsequently the blood was dropped into haemoglobin tube already containing N/10 HCL. The tube was shaken well for 10 minutes. Later on N/10 HCL was added drop by till the colors graduated tube match with the standard.

(b) **RBCs count or Total Erythrocyte count (TEC)**: - The erythrocytes were counted with the help of haemocytometer (Neubaur's Counting Chamber) by using Hayem's addition solution. The standard RBCs diluting pipette and a 12.00 dilution was used as done in the study of mammalian erythrocytes. The
count of red cells was done under high magnification in the five
groups, each of 16 small squares. The calculation was done by
following Formula.

\[ \text{RBCs Count per cubic mm} = 10,000 \times \text{Number of cells Counted in 80 smaller squares.} \]

(c) **WBCs count or Total Leucocytes Count (TLC):** The leucocytes
were counted with the help of haematocytometer (Neubaur’s
Counting Chamber) by using Turk’s solution.

The standard WBCs diluting pipette and dilution was used as done
in the study of leukocyte in mammals. The count of white cells was done
under high magnification in the four groups, each of 16 large squares.
The calculation was done by following Formula.

\[ \text{Total WBCs count per cubic mm} = \text{Number of cells counted in 80 smaller squares} \times \text{dilution.} \]

(d) **Clotting Time (CT)** – The blood was drawn in a capillary and the
stopwatch was started immediately. The capillary was broken in
portions and the watch was stopped when fine strands of fibrin
were seen between the broken ends. The stop watch was stopped
instantly and the clotting time was noted.

(e) **Prothrombin Time (PT)** - The calcium chloride solution was
taken in a test tube and placed in water bath. In a separate test tube
requisite quantity of reagent and plasma was taken and kept to gain temperature equilibrium. Requisite amount of calcium chloride solution was transferred to the plasma tube and the stopwatch was started instantly. The three solutions - reagent, plasma and calcium chloride, were mixed and the tube was left in the waterbath for 10 seconds. The tube was taken out and starts tilting intermittently. The watch was stopped instantly when jelly like fibrin strands appear before the clot.

(f) **ESR** (Erythrocyte sedimentation rate):- For determination of Erythrocyte sedimentation rate (ESR), the blood was mixed well and 10 mm. was drawn into the wintrobe tube. The tube was placed vertically undisturbed for 60 minutes. After 60 minutes the level of the column of sediment was noted as ESR.

(g) **PCV** (Packed cell Volume or Haematocrit):- PCV was estimated by ultra micro method, described by Nelson (1951). Plain capillary tubes of about 100 mm. length with an internal diameter of about 1 mm. were used. The blood was allowed to enter the tube by capillary action leaving at least 15 mm unfilled. On end of the tube was sealed by heating the tube rapidly on a fine flame. After centrifugation for 10 minute the PCV was calculated by using the formula:

\[
PCV = \frac{\text{Length of Red blood cells column}}{\text{Length of the whole blood column}} \times 100
\]
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(h) **MCH (Mean Corpuscular Haemoglobin):** - MCH values express the average haemoglobin content of a single red cell in Picograms (pg). The mean corpuscular haemoglobin is derived from Hb value of blood sample and Erythrocytes count by following Formula.

\[
MCH = \frac{\text{Hb in gm/100 ml of blood}}{\text{RBC in millions mm}^3} \times 10 = \text{Pg}
\]

(i) **MCV (Mean Corpuscular Volume):** - MCV, often used in place of diameter measurements, is the assessment of abnormalities in cell size. It is derived from Haematocrit value of blood sample and erythrocyte count. All these parameters were calculated as per method described by Nour-Eldrin (1993). The calculation was done by following Formula:

\[
MCV = \frac{\text{PCV/100 ml of blood}}{\text{RBC in millions mm}^3} \times 10 = \text{Fl}
\]

(j) **MCHC (Mean corpuscular haemoglobin concentration):** - Mean corpuscular haemoglobin concentration (MCHC) is calculated from two accurate and reproducible observations i.e. packed cell volume and amount of haemoglobin in 100 ml of blood. The calculation was done by following Formula:

\[
MCHC = \frac{\text{Hb in gm/100 ml of blood}}{\text{PCV/100 ml of blood}} \times 100 = \text{g/l}
\]
Biochemical studies -:

The estimation of serum bilirubin, acid phosphatase, alkaline phosphatase activity SGOT, SGPT and serum creatinine, blood urea was done by calorimeter using standard chemistry.

(a) **Serum bilirubin**: Estimation of serum bilirubin was be done by calorimeter method using standard kit.

(b) **Acid and alkaline phosphatase**: Estimation of Acid and alkaline phosphatase was be done by kinetic method using standard kit.

(c) **Serum Glutamyl oxaloacetic Transminase activity (SGOT)**:
Estimation of SGOT was done by auto analyzer using standard chemistry and by means of Kit (**kinetic method**).

\[
T - C \times 3 = \text{Result IU/dI}
\]

\[
T = \text{Test Value}
\]

\[
C = \text{Control Value}
\]

(d) **Serum Glutamyl Pyruvate Transminase activity (SGPT)**:
Estimation of SGPT was done by auto analyzer using standard chemistry and by means of Kit (**kinetic method**).

\[
T - C \times 5 = \text{Result IU/dI}
\]

\[
T = \text{Test Value}
\]

\[
C = \text{Control Value}
\]
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(e) **Serum Creatinine**: Estimation of Serum creatinine was done by using kinetic and end point method (**Jaffe’s method**) in computerized semi-autoanalyser.

(f) **Blood Urea**: Estimation of blood urea was done by Calorimeter method using standard kit (**Berthelot method**).

**Standard Deviation**

An attempt was made for comprehensive analysis of data collected for this research project. To assess the changes and to find out the statistical significance of the result obtained, **Student 't' test** was used. That test is the statistical test of choice for testing the significance of difference in this kind of study.

The standard deviation (SD) was used to determine the variation in the sample observation and have been calculated with help of the following formula:

\[
S_1 = \sqrt{\frac{\sum (x_1 - x)^2}{n_1 - 1}}
\]

\[
S_2 = \sqrt{\frac{\sum (x_1 - x)^2}{n_2 - 1}}
\]

\(S_1, S_2 =\) Standard Deviation of samples 1 and 2.

\(\sum (x_1 - x)^2 =\) Summation of squares of the deviation of each reading from the mean for the first sample.
\[ \sum (y_1 - y)^2 \] = Summation of squares of the deviation of each reading from the mean for the second sample.

\[ n_1 - n_2 = \] Total Number of observation for first and second sample respectively.

\[ n_1 - n_2 - 1 = \] Degree of freedom of the first and second sample respectively.

The Student 't' test was calculated with the help of the following formula and values obtained were compared with the table values given by Fisher ands Yates (1976) to find out the level of significance and analogue results for the comparison of the two.

Where \( x, y \) = means of first and second samples respectively.

\[ t = S \sqrt{\frac{\bar{x} - \bar{y}}{\frac{1}{n_1} + \frac{1}{n_2}}} \]

\( n_1, n_2 \) = number of first and second samples respectively.

\[ S = \text{Common Standard Deviation} \]

\[ S = \sqrt{\frac{1}{n_1 + n_2}} - \frac{\gamma((n_1 - 1)S_1^2 + (n_2 - 1)S_2^2)}{n_1 + n_2} \]

**Photomicrography**

Photographs were taken under Olympus trinocular microscope at a magnification of 100x, 400x and 1000x.