PART-II PHARMACOLOGICAL STUDIES

7. MATERIAL & METHODS
7-MATERIAL AND METHODS

In the preliminary Pharmacological investigations with various extracts prepared with benzene, ether, chloroform, acetone, alcohol and water, the alcoholic extract of the fruits was found to be most active. In Ayurvedic and Unani system of medicine, the drug is used mainly in the form of aqueous infusion. Therefore, for the present pharmacological investigations, alcoholic extract, aqueous extract and various chemical constituents isolated in major quantities from Withania Coagulans fruits have been used in the following form:

1) Alcoholic extract dried to constant weight of defatted fruits (WC-1).
   a) Water soluble fraction of the alcoholic extract (WC-1a)
   b) Water insoluble fraction of the alcoholic extract (WC-1b)
2) Total alkaloids (WC-2)
   a) Water soluble fraction (WC-2a)
   b) Water insoluble fraction (WC-2b)
3) Aqueous extract (1:5) of the fruits (WC-3)
4) Withanolides (m.p. 260-61°) identified as 3β-hydroxy-2,3-dihydrowithanolide F (WC-4).
Other constituents isolated in Crystalline form were not assessed pharmacologically because these could be obtained only in minor quantities and compounds like hydrocarbons are known to be pharmacologically inert.

Suspensions of appropriate concentration of WC-1, WC-1b, WC-2 and WC-2b were made in 2% gum acacia for oral use and in 1% sodium carboxymethylcellulose (CMC) for intravenous use. WC-3 was prepared by maceration with water using drug/menstruum ratio of 1:5. It was always prepared afresh. Unused portions of these preparations were stored in refrigerator. Suspension of Withanolide was made in 1% CMC and sterilised in boiling water for 15 min. For some cardiovascular studies, Withanolide was dissolved in absolute alcohol and diluted with saline before use. Unless otherwise stated, suspension of the withanolide was given by i.p. route in 5-10 mg/kg dose and other preparations were administered by intragastric route (p.o.) by gastric intubation in doses of 1 gm/kg of WC-1, 200 mg/kg of WC-2 and 5 ml/100 gm of WC-3 of body weight. Equivalent doses of vehicles were used in control experiments. The effects of solvent control were found to be insignificant and hence omitted from the text.
Animals: Albino rats and albino mice were procured from Haryana Agriculture University, Hissar and fed on Gold Mohur rat feed, a standard diet, manufactured by Hindustan Levers Ltd., Bombay. Other animals used were mongrel dogs, rabbits, guinea pigs and frogs of either sex.

7.1. CENTRAL NERVOUS SYSTEM

1) General behaviour (Irwin, 1964):

The general behaviour of Albino rats (100-200 gm) of both sexes was observed. The WC-1 and WC-2 were used in graded doses whereas WC-3 was used as 5 ml/100 gm of body weight by gastric intubation. WC-4 was given in graded doses by i.p. and i.v. routes. Drugs were administered to overnight fasted (water ad lib allowed) animals 30 min. before observations.

2) Analgesic Activity:

1) Thermal Stimulation Method:

The method used was of Davies et al, 1946; Dandiya and Menon (1963) using hot wire Technoanalgesiometer. The rats (100-200 gm) were used. The apparatus used for
the experiment (Technoanalgesiometer) has a nichrome wire which could be heated to the required temperature and maintained by means of the heat regulator. The current passing through the nichrome wire is indicated on the ammeter which directly gives the temperature of the wire. A jacket surrounds the nichrome wire and water circulated through it. The upper surface of the jacket serves as a platform on which the tail of the animal can be placed. The water circulating through the jacket prevents the platform from getting heated up. This ensures that only that portion of the tail which lies just above the hot wire is affected.

The temperature of the wire was so adjusted that the normal reaction time did not exceed 3 sec. The rat was kept in rat holder with only the tail portion remaining outside. The tail was placed on the platform so that the middle portion of the tail remained just above the hot wire but without touching it. When the animal responded with a sudden and characteristic flick, the reaction time was noted. For testing the analgesic effect, the reaction time was noted before and after administering the drugs.
Fig. 22: Monsanto hardness tester.
ii) Caudal Compression Method:

In the present investigations, a simple modification of Green et al (1951) method standardised in this laboratory has been used to measure the gradual rise in pressure. In this method 'Monsanto Hardness Tester', an instrument designed by Monsanto Chemical Co. Ltd. (Rawlins, 1977) (Fig. 22) for testing the hardness of tablets, was used for measuring the pressure causing struggle or squeak. The instrument measures the pressure in Kg. graduated in 0.5 Kg. and reliable to read 0.25 Kg.

The pressure was measured at the middle of the tail by placing the tail of the rat between the spindle and anvil. When the tail was just held between the spindle and anvil, reading of the pointer in the scale was adjusted to read zero and then the pressure increased gradually by turning the knurled knob. Reading on the pointer for estimating the pain threshold was taken when rat responded by struggle. The pressure was then released by turning back the Knurled Knob instantly.

This method of measuring pain threshold was standardised for its sensitivity, reproducibility and
reliability by repeated tests using morphine sulphate and methadone hydrochloride as control.

For assessing the analgesic effect of the extracts and the withanolide, pain threshold causing struggle was measured with Monsanto Hardness Tester before treatment and after 30 min. and 60 min. of drug treatment.

3) Anticonvulsant Effects:

WC-I, WC-2, and WC-4 were tested for anticonvulsant effect on adult albino rats as follows:

1) Protection against Maximal Electroshock Seizures (MES): Method of Toman and Everett (1964) was used. Electric, shock seizures were induced by delivering a current of 150 mA through corneal electrodes for a period of 0.2 seconds from a convulsometer. Animals which showed tonic extension of the hind legs were selected and given rest overnight. Test drugs were given to groups of 6 rats and electroshock was given after 1 hr. Phenobarbitone (30 mg/kg i.p.) was used for control group.
Animals were considered 'protected' if the drug prevented appearance of the hind limb tonic extension component of the seizure.

ii) Pentylene tetrazol (PTZ, Metrazol) convulsions:

For chemoconvulsions, method of Prasad and Malhotra (1968) and Soaje-Echagae and Lin (1962) was used. Convulsions were produced in adult rats with metrazol with doses of 70 mg/kg and 112 mg/kg i.p. The convulsions began with jerks of the head and body consisting chiefly of clonic contractions. The seizures ended fatally or were followed by depression and recovery finally took place. Test substances, WO-1, WO-2, WO-4 and phenobarbitone were given 45 minutes before injecting metrazol. The criterion for anti-convulsant activity was the prevention of the tonic extension of the hind legs. Tremors were disregarded. Observations were made for 60 minutes after giving PTZ 112 mg/kg and for 24 hrs. in case of 70 mg/kg PTZ.

iii) Audiogenic seizure test (Plotnikoff and Green, 1957):

The protective effect of WO-1, WO-2, was tested by using Techno Audiogenic Seizure Test Chamber. Albino rats
weighing between 80—100 gm were used. Rats, one at a
time, after placement in the chamber, were exposed to the
auditory stimulus for a period of ninety seconds. Stimulus
was produced by two door bells in the chamber. Ten rats
in which convulsive response was produced were selected.
After an overnight rest, the animals were treated with
the test drugs and after 60 min. of drug administration
were subjected to auditory stimulus as before. The
response was graded as follows: Running, jumping,
circling, convulsing and immobility (Catalepsy) following
a convulsive seizure and the animal scored one point for
each response if present.

The effect of the drug was evaluated by using
each group as its own control and determining its response
to a non-medicated solvent on the day before treatment.
The difference between scores on control and treated days
was then used as the measure of protection afforded by
the drug i.e. Percent protection.

\[
\text{Percent protection} = \frac{(C-t) \times 100}{C}
\]

Where \( C \) is control score and \( t \) is treated score.

The dose which reduced the total response to fifty
percent of the control value \( (PD_{50}) \) were estimated by
the technique of Miller and Tainter (1944).
4) **Protection against metrazol and amphetamine toxicity** *(Trepamier et al. 1959):*

The effect of WO-2 and/or reserpine was studied on amphetamine and metrazol induced toxicity in aggregated rats. Methyl-amphetamine sulphate (20 mg/kg) and metrazol (70 mg/kg) were used. Test drugs were administered 1 hr. and reserpine 4 hrs. prior to the administration of the stimulants. The number of dead animals was counted after 24 hours.

5) **Hypnotic potentiation activity** *(Jacobsen, 1964):*

Potentiation of pentobarbitone sleeping time was studied in each group of 25 rats. In control animals sleep was induced with 30 mg/kg of pentobarbitone sodium injected i.p. After the rats had fallen asleep they were laid on their backs. Time of onset of hypnosis was recorded as the time required for loss of righting reflex. The waking time was taken as that at which the rat righted itself three times within a minute when placed on its back. For testing the drugs, the animals were pre-treated with WO-1, WO-2 60 min. and WO-4 30 min. before the administration of pentobarbitone sodium i.p.
and the sleeping time was recorded.

6) **Body Temperature:**

The effect of WO-I, WO-2 and WO-4 was assessed on the rectal temperature of rats of both sexes. Overnight fasted rats were used. Experiments were carried out at room temperature (22-25°C). Rectal temperature was recorded with a rectal thermometer lubricated with liquid paraffin. Rectal temperature recorded before and at 15, 30, 60, 90 and 120 min. after the administration of test drugs.

7.2. **LOCAL ANAESTHETIC ACTIVITY**

Local anaesthetic effect of WO-1a(5%), WO-3 (1:5) and WO-4(1% in CMC) was tested by the 3 methods described by Camougis and Takman (1971) using 2% xylocaine as control drug.

1) **Surface anaesthesia:**

Using rabbits of either sex with eye lashes cut, 0.2 ml of each drug was instilled into the left conjunctival sac of each rabbit keeping right eye as control. Corneal reflex was tested at 5 min. interval for 30 min. by touching the corneal surface with a glass
rod with blunt end. If the eye is anaesthetised, the touch of the rod does not elicit the corneal reflex (blinking).

2) Nerve Block Anaesthesia (Sciatic Nerve Block):

Effect of the drugs for conduction anaesthesia was tested in rats (125-175 gm) by injecting 0.2 ml of each drug underneath the skin of the junction of the biceps femoris and gluteus maximus. Observations of the walking behaviour and change in digits of foot were recorded at 5 min. intervals for 30 min. Successful block is characterised by dragging the leg and inability of the animals to use the leg in walking up the inclined surface. In addition, in blocked leg the digits of the foot are close together which in normal digits are wide apart.

3) Infiltration Method:

For this, shaved the skin on the back of guinea pigs (300-400 gm). Injected 0.1 ml of the test drugs with NO 26 needle intradermally causing circular wheel and marked wheel with pencil. Three wheels were made on each side. The marked area was pricked 6 times with
a needle at 10 min. intervals for 1 hr. to see the presence or absence of twitching reflex.

7.3. **ANTH-INFLAMMATORY STUDIES**

Anti-inflammatory effects of W0-1, W0-2, W0-3 and W0-4 were studied by the following methods.

**Measurement of hind-paw oedema of rats:**

The paw volume of hind paw of rats upto a fixed mark at the level of lateral malleolus was measured plethysmographically by causing displacement of mercury by slight modification of method as described by Satyavati et al (1969). For this, a 10 ml syringe containing mercury mounted on an adjustable stand was connected to a 3 ml graduated pipette with a transparent polyethylene tube and fixed the pipette on another adjustable stand. The lateral malleolus of the rats was marked with a skin pencil before dipping the paw into syringe. The rat was lightly anaesthetised with ether and the paw was dipped upto the mark on the lateral malleolus which was made to coincide with a prefixed line kept constant on the syringe. The level of mercury was brought to the level of this line by adjusting the height of the pipette and the reading was taken. The difference in the readings
before and after injection of the phlogestic agents gave
the difference in the paw volume due to oedema.

1) Acute Oedema:

Acute oedema of hind paw of adult albino rats
(150-200 gm) of either sex was produced by injection of
0.1 ml each of 1% Carrageenin, 3% formalin, 0.1% 5-HT and
2% egg albumin in normal saline under the plantar
aponeurosis (Sofia, 1978). The paw volume (in ml) of the
treated rats was measured as described above before
and at various intervals after the administration of the
phlogestic agent. Difference in paw volume before and
after the phlogestic agent was designated as oedema volume.
Average percentage inhibition of the oedema was calculated
using the formula:

Average % inhibition = \( \frac{(VC-Vt)}{VC} \times 100 \), where

VC: Average paw volume in control group,
Vt: average paw volume of treated group.

Average percent increase of oedema was calculated using
the formula:

Average % increase in volume = \( \frac{(VP-Vt)}{Vt} \times 100 \), where

VP: Average paw volume after phlogestic agent,
VI: Average initial paw volume before the phlogestic agent.

Rats used in the experiment were kept fasting overnight. Test drugs were administered orally 1 hr. before inducing inflammation or by i.p. route 30 min. before inducing inflammation. Control: rats received equivalent amount of the vehicle.

2) Formalin Induced Arthritis:

For subacute oedema, 0.1 ml of 3% formalin was injected to each rat under the plantar aponeurosis on 1st and 3rd day of the experiment. The drugs were administered once daily for 10 days starting one day before injecting the phlogestic agent. Measurements of the paw volume were made daily for 10 consecutive days as the maximum oedema was observed after 9th or 10th day in control animals.

For histopathological examination, on the 10th day the rats were deeply anaesthetised with ether, the inflamed paw were cut and stored in 10% formal saline. Histopathological studies were carried out after staining the slides with haematoxylin and eosin solutions.
3) Granulation Tissue Formation:

Granulation tissue formation was measured by the implantation of cotton-wool pellets (Meyer et al, 1953). Cotton pellets, 10 mg each, were sterilised at 180°C for 20 min. in hot air oven. These were implanted bilaterally both in pectoral and groin regions. Pellets were implanted through small incisions into loose connective tissues. Care was taken to place the pellets in the subcutaneous pockets in such a way that connective tissue surrounded it on all the sides. The skin incisions were closed with thread. The pellets and their attendant granulation tissue were removed after having been in place for 120±4 hr. Each granuloma was dissected out of the surrounding loose connective tissue and weighed. Infected granulomas were rejected. The average values were determined as follows:

The initial dry weight of cotton pellet was subtracted from the total wet weight of each granuloma removed, giving the wet weight which included the fluid absorbed by the cotton. The average weight of the granuloma from each rat was determined and the mean of these averages ± S.E. of mean was calculated for each group.

The test substances were administered once daily.
starting one day before starting the experiment and continued for 5 consecutive days. WC-1, WC-2, and prednisolone were administered by intragastric route whereas the withanolide, hydrocortisone acetate and phenylbutzone by i.p. route for comparison. The implantation of cotton pellets and removal of the granulomas was carried out under ether anaesthesia.

7.4. CARDIOVASCULAR STUDIES

WC-1, WC-1a and b; WC-2, WC-2a and b, WC-3 and WC-4 were tested for cardiovascular effects. Further a 1% solution of withanolide was made in absolute alcohol and diluted with saline before use for dog experiments and rat hind limb preparation. For other experiments, 1% suspension of withanolide in 1% CMC was used. Unless otherwise stated, the drugs were administered through femoral vein. Following studies were carried out.

1) Blood Pressure and Respiration (Ghosh, 1971):

1) Mongrel dogs (12-15 kg) anaesthetised with 30 mg/kg pentobarbitone sodium were used for recording blood pressure and respiration. Mean arterial blood pressure
was recorded from a common carotid artery by a mercury manometer and respiration from trachea by means of Brodies tambour. Carotid baroreceptor reflex was tested by occluding both the common carotid arteries for 30 sec. The effect on blood pressure and respiration was also observed by introducing the drug by intragastric route.

ii) Rats (250-300 gm) anaesthetised with urethane (1.5 gm/kg) and rabbits anaesthetised with pentobarbitone sodium (30 mg/kg i.v.) were used for recording blood pressure. Blood pressure was recorded by a Condon’s manometer from Common Carotid artery. Drugs were administered through jugular vein.

2) Perfused Frogs Heart:

For this study adult common Indian frogs (100-200 gm) perfused heart was set up as described by Burn (1952a) using frog’s Ringer. Heart was tested for its normal physiological functions with adrenaline and acetylcholine. After that various doses of the extracts, alkaloids (including water soluble alkaloid) and suspension of the withanolide were tested before and after atropine
and propranolol. Effects on cardiac rate and amplitude was recorded before and after administration of various extracts. Records of contractions were taken using frontal writing lever on a smoked drum.

3) Perfused Hind Limb Preparation:

Perfused hind limb preparation of albino rat was set up as described by Burn (1952 b). Rats were anaesthetised with pentobarbitone (30 mg/kg i.p.). The animals were kept in supine position and lower abdominal aorta was exposed by paramedian suprapubic incision. The abdominal aorta was cannulated which was tied with a ligature to the body of the rat and the upper half of the animal was cut off. Both the hind limbs of the animal were placed in a glass funnel resting on stand ring. The canula in the aorta was connected through a tubing to a Mariotte bottle containing ringer solution and kept at a height of 30-50 cm.

4) Langendorff's Preparation (Langendorff, 1895):

Rabbit isolated heart preparation was set up as recorded by Langendorff (1895). The adult rabbits (1.5-2.5 kg) of either sex were used. The animal was sacrificed by stunning and cutting the throat to
exsanguinate it. The heart was carefully removed along
with half an inch of aorta. It was then placed on oxygen-
ated warm Ringer Locke's solution at 37±0.5°. The heart
was gently squeezed to remove blood from cavities and
to prevent clotting inside the coronaries. The aorta
was tied in position over the tip of the glass cannula
in the Langendorff's apparatus. A suture was taken
through the apex of the ventricle which was led over a
system of pulleys and connected to Starling's lever for
recording the contraction on a smoked drum. The coronary
flow as measured at every minute interval before and
after different doses of drugs. The temperature was
maintained at 37±0.5° and Ringer Locke's solution was
oxygenated continuously.

5) ECG Studies:

ECG studies were conducted on dogs anaesthetised
with pentobarbitone sodium (30 mg/kg IV). Records were
made on a single channel ink recording electrocardiograph.

7.5. DIURETIC EFFECT:

Burn's method as recorded by Heller and Urban(1935)
was used for finding out the diuretic effect. Rate of
both sexes (100-150 gm) were used. The animals were fasted overnight before the experiment but allowed water ad libitum. For the estimation of effect, groups of 8 rats were used. Test substances were administered in volume of water load which was used as 5% of body weight (at body temperature). Four rats were placed together in the metabolic cages. Cross over test was carried out after 2-3 days of rest. Urine volumes were recorded at 1 hr. interval for 5 hr. and expressed as percentage of administered load to 16 rats (Reizi et al, 1955).

7.6. PROTECTIVE EFFECT AGAINST CARBON TETRACHLORIDE INDUCED HEPATIC DAMAGE:

Protective effect of W0-3, W0-4 and hydrocortisone (for comparison) was evaluated in CCl4 induced acute hepatotoxicity in adult albino rats (150-200 gm) of either sex. The protective effect was assessed by:

1. Observing Pentobarbitone induced sleeping time (Maest et al, 1958)
2. Determination of SGOT and SGPT (Wilkinson, 1962)
3. Histopathological examination of hepatic tissues.

Liver damage was induced with fresh mixture of equal volume of CCl4 and olive oil (Bortolla Olive Oil,
Italica) by giving 3 injections at 3 days interval i.e. on 1st, 4th and 7th day in doses of 2 ml/kg of CCL\textsubscript{4} by i.p. route. Test drugs were administered once daily beginning one day prior to the experiment (0 day) and continued for 10 consecutive days. WC-4 and hydrocortisone were given by i.p. route and WC-3 orally.

For taking the samples of blood for SGOT/SGPT determinations and livers for histopathological studies, the animals were anaesthetised with ether on the 10th day, thoracoabdominal incision was made and rats were exsanguinated by intracardiac puncture. Serum was separated for the determination of transaminases.

Total hepatectomy was then performed. The livers were preserved in 10\% formal saline for gross examination and histopathological studies.

Pentobarbitone induced sleeping time was observed for detection of hepatic damage as recommended by Flaa et al (1958). For this control sleeping time was determined before administering hepatotoxic substance and then on the 9th day of injecting first dose of hepatotoxic substance. Sleeping time was determined by injecting 30 mg/kg i.p. of pentobarbitone sodium as described under C.N.S. studies.
SGOT and SGPT were determined by the method described by Wilkinson (1961). For histopathological studies, liver tissues were prepared by the standard technique, 5 micron thick sections were cut and stained with haematoxylin and eosin. These were examined under both low and high powers of microscope.

7.7. ISOLATED TISSUES

1. Smooth Muscles:

1) Isolated guinea pig ileum:

Overnight fasted guinea pigs of either sex weighing 250-500 gm were sacrificed by a blow over the head and bled out. Strips of ileum approximately 3 cm long were set up under a resting tension of 1 gm in 30 ml organ bath containing Tyrode solution maintained at 37±0.5° and bubbled with oxygen. Contractions were recorded via an isotonic frontal writing lever on a kymograph.

Tissues were dosed with adrenaline or test drugs allowing a 30-60 sec. contact time with each drug concentration. The effect of priscoline and propranolol
on the response was investigated. The drugs were tested for their spasmolytic effect against spasmogenic drugs like acetylcholine, histamine and barium chloride.

ii) Isolated rabbit intestine:

Effect of test drugs were observed on ileum of the rabbit (1-2 kg) of either sex fasted overnight. Animals were sacrificed by stunning and 3-4 cm pieces of intestine were set up under a resting tension of 1 gm in 30 ml organ bath containing Tyrode solution maintained at 37±0.5° and oxygenated continuously. An interval of 6-10 min. was maintained between two successive administration of test drugs. The effect of priscoline and propranolol on the responses was also investigated.

iii) Isolated uterus of albino rats:

Female albino rats (150-200 gm) were sacrificed by a blow over head and bled out. Uterus was set up in 30 ml organ bath containing modified Ringer solution (de Jalon's solution) at 25-36° anywhere in the range at constant temperature and oxygenated continuously. Spasmolytic activity of test drugs against acetylcholine
were recorded via an isotonic frontal writing lever.

iv) Isolated rats intestine:

Effects of various test compounds were assessed on adult rat's isolated intestine using de-Jalon's solution in a 30 ml bath maintained at 37±0.5°C and bubbled with oxygen continuously. Animals were fasted overnight and sacrificed by a blow over the head and bled out. Strips of intestine 2-3 cm long were used. Responses were recorded via an isotonic frontal writing lever on a kymograph drum. Spasmolytic effect against acetylcholine, histamine and barium chloride induced spasm was recorded. The effect of priscoline and propranolol on drug responses was also tested after the pre-treatment of tissues with these blockers.

2) Striated Muscle:

Adult frogs (100-200 gm) were pithed. The rectus abdominis muscle was isolated and mounted in an organ bath with a capacity of 30 ml containing frog's Ringer maintained at room temperature and continuously oxygenated. After relaxing the muscle for 1 hr. initially, contractile response of acetylcholine was recorded.
isotonically by a frontal writing lever. Submaximal dose of acetylcholine was repeated in the presence of different doses of test drugs.

7.8. **ANTIBACTERIAL ACTIVITY OF WC-4:**

Antibacterial activity of WC-4 was tested by the disc diffusion method described by Aggarwal (1974).

Discs of 4 mm in diameter were cut out with a punch from Whatman Filter Paper No.1 and sterilised at 160° in hot air oven for 1 hr. The sterile discs were separated on a wire mesh and kept in a 150 mm sterile petridish so that each disc was 2 mm apart. On each disc 0.01 ml of Withanolide solution in methanol (equivalent to 200 μg disc) was dropped using a tuberculin syringe. Discs were dried in an incubator at 37°C for 30 min and stored in sterilised bottles.

Lab Lemco agar medium was poured in sterilised 100 mm flat bottomed petridish to a thickness of 4-5 mm. Overnight growth of standard reference strains of *Staphylococcus aureus* and *Escherichia coli* were diluted to 1:100 and sterile swab sticks were soaked in this suspension. It was inoculated in the whole plate to
give semi-confluent growth. One medicated disc was placed in each of three inoculated plate of each bacteria. Plates were then kept at 4°C in cold place for prediffusion of drug for 30 min. before incubating at 37°C for 16-18 hrs. Zones of inhibition were measured.

7.9. **ANTI TUMOR STUDIES**

Antitumor tests of the withanolide (W0-4) have been carried out by Dr. Mathew Suffness, Acting Chief, Natural Products Branch of NIC, Bethesda, Maryland, U.S.A. against the P388 lymphocytic leukemia (06Ps31) using six CDF1 male mice per dose level in 5 groups. The material was administered intraperitoneally as a suspension in hydroxypropyl cellulose (Klucel). The treatment schedule was once every five days (qD 1x5), starting day 1, with a total of five injections at dose levels ranging from 11.25 to 180 mg/kg per injection. The Ps31 system is a survival test system where the animal body weight change of the test group is subtracted from that of the control animals in grams and the activity is expressed in % T/C where an increase in survival of treated animals/controls resulting in a T/C ≥125% is considered inactive.
7.10. ACUTE TOXICITY STUDIES

Acute toxicity of alcoholic extract and alkaloids was carried out on albino rats of either sex (100-150 g). The drug was given p.o. and i.p. in graded doses to groups of 5 animals. The animals were fasted overnight prior to being used but immediately after the administration of drug, these were returned to the cages containing food and water. They were kept under observation for 48 hours. Mortality, if any, was recorded.

Acute toxicity of withanolide was tested under the above conditions using mice (18-25 gm) and the withanolide suspension was administered by i.p. route.