Name of Unit	Screening Models of ANS
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Learning Outcome of Module 03

LO	Learning Outcome	Course Outcome Code
LO 1	To remember and understand the preclinical screening models used to evaluate drugs acting on the autonomic nervous system (ANS).	BP810.3
LO 2	To understand and explain the screening models for drugs such as sympathomimetics, sympatholytics, parasympathomimetics, and parasympatholytics.	BP810.3
LO 3	To apply suitable screening methods to study the pharmacological effects of skeletal muscle relaxants, drugs acting on the eye, and local anaesthetics.	BP810.3
LO 4	To analyze the pharmacological responses obtained from ANS activity screening models.	BP810.3

Content Table

Topic
Preclinical Screening Models for ANS Activity • Sympathomimetics • Sympatholytics • Parasympathomimetics • Parasympatholytics. • Skeletal muscle relaxants. • Drugs acting on eye. • Local anaesthetics.

SYMPATHOMIMETICS

Sympathomimetics are the agents that mimic the activity of adrenaline (epinephrine) in the periphery. Adrenaline is secreted by adrenal medulla in mammals. Nor-adrenaline (norepinephrine) is a predominant neurotransmitter of the sympathetic system, which acts on sympathetic receptors (α and β). The sympathetic receptors are mainly found in heart, blood vessels, bronchi, uterus, GI tract, and eye. Stimulation of sympathetic nerves by adrenaline or electrical stimuli may trigger the following responses: heart rate acceleration, blood pressure stimulation, mydriasis in the eye, increased stroke volume in the heart, coronary arteries dilatation, constriction of pulmonary vessels, bronchial muscle relaxation, inhibition of gastric secretion, gastrointestinal sphincters contraction, and stimulation of the uterus.

The animal models that are based on these sympathetic responses are summarized in the following table :

Table 19.1: Animal models used for screening sympathomimetics

Sr. No.	Screening Models	Description
I	**In Vivo Models**	
A	Bioassay of adrenaline by monitoring blood pressure in dogs	Intravenous (femoral vein) injection of adrenaline increases blood pressure due to activation of sympathetic receptors (α and β).
B	Rabbit eye model	Topical application of adrenaline (0.1%) or sympathomimetic solution dilates the pupil by activation of β_2 receptors in the eye.
C	Cat spleen model	Sympathomimetics cause contraction of the spleen and trigger the release of noradrenaline. The drug is administered into the splenic artery through a cannula, and the sample is collected for estimation of noradrenaline content.
D	Spinal cat model	Cats are used to assay adrenaline by measuring blood pressure. The posterior pituitary of the cat is removed, and the procedure similar to the dog blood pressure model is followed.
	In-Vitro Models	
A	Bioassay of adrenaline using rabbit intestine	The intestinal duodenum is used in this model. Gastrointestinal smooth muscle contains β_2 receptors, which are responsible for GI relaxation during

Sr. No.	Screening Models	Description
		sympathetic stimulation. A matching bioassay technique is used to determine the potency of the test substance.
B	De Jalon's Method on rat uterus	A virgin female rat is used to isolate the uterine horn. Administration of sympathomimetics inhibits the contractile response produced by carbachol (0.75 µg/ml). The K_{250} values (percent inhibition) of sympathomimetics are calculated.
C	Isolated frog heart preparation	This model is used to determine the effect of sympathomimetic drugs on heart rate and force of contraction using an isolated frog heart preparation.

IN VIVO MODELS

A. Bioassay of adrenaline by monitoring blood pressure in dogs

Measuring the blood pressure is the main parameter to evaluate adrenaline and other sympathomimetics. When adrenaline is injected by i.v. route to the animals, blood pressures (systolic and diastolic) elevate rapidly to a peak due to sympathetic stimulation and then rapidly decrease, below the base line before returning to the baseline (biphasic response). The acceleration of the heart (pulse rate) may be reversed during the episode due to the compensatory action of the vagus nerve (parasympathetic) on the heart. This bradycardia is eliminated after vagotomy or atropine injection. The vasopressor action is directly proportional to the amount of adrenaline which is administered. Therefore the bioassay of sympathomimetics or adrenaline may be assayed by examining vasopressor response in atropinized animal like dogs. Cats and rodents can also be used to monitor the blood pressure response of adrenaline or other sympathomimetics

Method:

Healthy dogs are selected and anesthetized with barbiturate (25 mg/kg i.v.) and acclimatized for blood pressure monitoring. Blood pressure is measured by a kymograph after cannulating carotid artery with the arterial cannula and connecting the same with a mercury manometer. The kymographic recording is of animal models for screening antihypertensive agents. The standard solution of adrenaline (4% w/v in 0.1 HCl) is prepared and different dilutions of adrenaline are prepared by diluting it with saline. A suitable

diluting concentration is 1 : 10,000. Test sample solution is also prepared in saline. Animals should be given atropine sulphate (1 mg/kg i.v.) to eliminate vagal action and vagal paralysis is tested by injection of acetylcholine chloride (10 μg/kg) or vagus electrical stimulation. There will be no fall in BP in atropinised animal after electrical stimulation or acetylcholine injection. Adrenaline is injected into the femoral vein through the venous cannula and two successive responses of the same dose of adrenaline are recorded. The observation of similar response after administration of the same dose of adrenaline indicates that there is no variation in B.P. and that the animal is now ready for the assay. Then the test and the standard samples are given in alternate till both produce similar rise in blood pressure. Test and standard are injected at intervals of five minutes.

Interpretation: Sympathomimetics raise the blood pressure. The potency of the test sample is estimated by comparing the rise in blood pressure of the test sample with that produced by the standard preparation of adrenaline.

B. **Rabbit eye model:**

The iris of eyes is composed of two types of muscle fibres, the circular and the radial. The circular muscles are controlled by the parasympathetic system and the radial ones are innervated by sympathetic system. The stimulation of sympathetic and parasympathetic nerves produces mydriasis and miosis effect respectively and their paralysis produces opposite effects. Local administration of sympathomimetic drugs on eye dilates the pupil. They activate the β receptor on the eye and cause the ciliary muscle relaxation and produce mydriasis effect.

Method:

Healthy young rabbits (2–3 kg) are used in this model. Animals are divided into different groups: control (vehicle treatment), standard adrenaline treatment and test group (test sympathomimetic treatment). A 1% adrenaline hydrochloride solution is used as a standard drug & applied topically to their respective groups, and measured the pupil diameter. Calculate the mean diameter in each group.

Interpretation: Mean pupil diameter of standard and test groups are compared with the control group. The topical administration of adrenaline or sympathomimetics increases the pupil diameter and produce mydriasis effect in the eye (Bumker and Sears 1974).

C**. Cat Spleen model**

The model used for the screening of substances that affect the release of adrenergic transmitter from the sympathetic nerve. Sympathomimetics contract the spleen and increase the transmural pressure in the splenic artery. The contraction of spleen is measured by collecting spleen venous outflow at different transmural pressures. The sympathomimetic effect is analyzed with reference to adrenaline.

Healthy cats (2–4 kg) are used in this model. Cats are anesthetized by a suitable anesthetic agent. The animal is dissected to expose spleen. The vascular connections are cut between the spleen and omentum. The splenic nerves are cut to avoid the release of catecholamine from other sites. The adrenal glands are also removed to avoid the release of pressure amines. Around the portal vein, ligature is applied. Heparin is administered intravenously to prevent blood clotting. The abdominal region is filled with warm paraffin and aerated with a carbogen (a mixture of 95% O_2 and 5% CO_2). Vehicle/adrenaline/test sympathomimetic is injected into the splenic artery to contract spleen and to release the transmitter. Electrical stimulation can also be applied around the ligature for sympathetic stimulation. Then venous blood is collected by cannula. Blood samples are collected in chilled silicon coated centrifuged tubes. Blood sample is collected during the cessation and stimulation period. Nor-adrenaline is measured by scintillation counter for radioactivity measurement

Similar to in vivo method cat spleen is isolated and kept in plethysmograph filled with liquid paraffin. The isolated spleen is perfused with Krebs solution (7–15 ml/min) at 37°C and aerated with carbogen. 5 % dextran is added in the perfusion solution to generate osmotic pressure. Ascorbic acid (25 µg/ml) is also added to prevent nor-adrenaline oxidation. The sample is collected after starting the perfusion and noradrenaline content is estimated (Brown and Gillespie, 1957).

Interpretation:

The nor-adrenaline content after administration of adrenaline or test sympathomimetics are compared with the vehicle treated value. The sympathomimetics increase the release of noradrenaline. The different doses of the test compound are used to compare with standard.

<u>IN VITRO MODELS</u>

A. **Bioassay of adrenaline using rabbit intestine.**

Adrenaline β receptors are found in the intestinal smooth muscles. Activation of sympathetic β receptors

relaxes the intestine via cAMP pathway. Rabbit duodenum plex nerve are used in this model.

Method:

A healthy rabbit is euthanized. Rabbit is sacrificed and dissected to expose the abdominal region. A small piece of duodenum is suspended in the organ bath containing Tyrode's solution. It is aerated with carbogen (95% O_2 + 5% CO_2). Inclusion of adrenaline is made and response is recorded by kymograph. The standard preparation of adrenaline is also prepared in Tyrode. The responses are recorded by isotonic transducer. The unknown sample is tested to assess adrenaline (β) activity.

2 µg/ml, 4 µg/ml is administered in an increasing order upto ceiling effect with proper washing interval. Select the submaximal dose of adrenaline, administer it to be organ bath and take a response. The test sample is administered and matched with the standard. Adrenaline relaxes the duodenum and the same property is taken upto consideration for finding out the potency of the test sample. Determine the dose of test solution producing the same response by a sequence of matching technique (Ghose, 2008).

B. **De Jalon's Method on Rat's Uterus:**

This method is first demonstrated by De Jalon et al. (1945). The parasympathomimetics like carbachol contracts the uterus, which is inhibited by adrenaline. Sympathomimetics relax the uterus by activation of β_2 receptor.

Method: A virgin female rat (100–150 g) is used in this method. The rat is sacrificed to isolate the uterine horns. Uterine horns are ligated and suspended in a mammalian organ bath containing modified Ringer solution that contains NaCl 9.0 gm, $CaCl_2$ 0.06 gm, KCl 0.45 gm, Dextrose 0.5 gm, $NaHCO_3$ 0.5 gm, and distilled water 1000 ml and maintained at 37°C with aeration. The modified Ringer solution is used to abolish the rhythmic contraction of the uterus. Two identical contractions for 30 sec with carbachol (0.75 µg/ml) are recorded. The standard solutions of adrenaline and test preparation are prepared. Three to five different doses of standard adrenaline and test is selected for concentration response curve. After washing the tissue preparation, adrenaline/test drug is administered with carbachol to record the response. Each dose-response is taken after 3 washings. Percentage inhibition is calculated against carbachol alone (100%). The IC_{50} value of adrenaline and test drug is calculated by concentration response curve. The potency of test drug is compared with adrenaline (Ghose, 2008).

C. **Isolated frog heart preparation:**

Isolated heart preparation is used to evaluate the sympathomimetic activity on the heart. The

sympathomimetics like adrenaline and isoprenaline increase the heart rate and force of contraction due to activation of β_1 receptor on heart, while noradrenaline administration has little effect on heart rate because noradrenaline generally acts on α receptor.

Method: A healthy frog is sacrificed by pithing and dissected to expose the heart. Sinus venosus is cannulated using a glass cannula and secured with thread. Then the heart is isolated along with the cannula and perfused with Frog Ringer through the sinus venosus. A curved needle is inserted in the apex and attached to a kymograph force transducer and physiograph are also used in place of the lever device and it is connected to the heart. After recording baseline basic heart rate adrenaline (1 μg/ml) test sympathomimetics are administered and recorded the response. Sympathomimetics accelerate the heart rate. Observed heart rate is compared with basal heart rate (Bajpai and Norlhapati 2014, Smith 1953). However, this method is now obsolete due to ethical constraints.

SYMPATHOLYTICS

The Sympatholytics are the agents that inhibit the action of the sympathetic nervous system by blocking the sympathetic receptors (α and β). The sympatholytics e.g. phentolamine, prazosin, propranolol, atenolol, etc. are clinically used for the treatment of cardiovascular diseases. They decrease the blood pressure and heart rate, relax vascular smooth muscle, inhibit the mydriasis effect in the eye, and inhibit the relaxing effects in tracheal chain induced by sympathetic system activation (Brunton et al., 2006).

MODELS FOR SCREENING SYMPATHOLYTICS

The models for screening sympatholytics (Table 20.1) are based on the inhibitory action of sympathetic stimulation. The animals like dogs, cats, rabbits, rats and guinea pigs are generally used to evaluate sympatholytic action.

Table: Animal Models Used for Screening Sympatholytics

Sr. No.	Screening Models	Description
I	**In Vivo Models**	
A	Adrenergic antagonism in the rodent's eye	Sympatholytics block the action of the sympathetic system (mydriasis effect) in the eye. Rodents such as rats and mice are used. After 30 minutes of subcutaneous (s.c.) injection of the test drug, a sympathomimetic is administered and the pupil diameter is measured and compared with the control (vehicle).
B	Blood pressure	Anesthetized male mongrel dogs are used in this model. Administration of

Sr. No.	Screening Models	Description
	response in dogs	sympatholytics decreases blood pressure by blocking α and β adrenergic receptors.
C	Cardiovascular response in rats	Administration of sympatholytics in albino rats decreases blood pressure and heart rate. These agents also inhibit the cardiovascular response produced by sympathomimetic drugs.
D	Nictitating membrane prolapse in cats	Cats are used in this model. Administration of sympatholytics produces relaxation of the nictitating membrane due to blockade of sympathetic activity.
II	**In Vitro Models**	
A	Isolated frog heart preparation	This model is used to determine changes in heart rate using an isolated frog heart. Sympatholytics such as propranolol (200 μg/ml) reduce heart rate by blocking β_1 receptors on the heart or by a membrane-stabilizing effect.
B	Isolated aortic muscle strips	Aortic muscle mainly contains α_2 receptors. Sympathetic stimulation causes contraction of the aorta, whereas blockade of α_2 receptors relaxes the aortic muscle. Rabbits, cats, and rats are commonly used to isolate the aortic muscle. The percent inhibitory response of the test drug is recorded against noradrenaline-induced contraction.
C	Isolated guinea pig tracheal chain	This model is used to determine β_2 agonistic and antagonistic activity. Stimulation of β_2 receptors relaxes tracheal muscle, which is inhibited by β_2 antagonists. Percent inhibition of the test drug is recorded against isoprenaline-induced relaxation of the guinea pig tracheal chain.

IN VIVO MODELS

A. **Adrenergic antagonism in rodent's eye:**

Sympathomimetics like noradrenaline, adrenaline, and isoprenaline induce the mydriasis. This effect is blocked by α and β blockers. α-blockers (e.g., prazosin, phenoxybenzamine) block the action of noradrenaline, α and β blockers (e.g., labetalol, carvedilol) block the action of adrenaline and β blockers (e.g., propranolol, atenolol, metoprolol) block the action of isoprenaline.

Method:

Albino rats (120-150 g) or albino mice (25-30 g) are employed in this model. Experimental animals are divided into control (vehicle) and test group (test preparations) having 4-6 animals in each. Vehicle/test

drugs are administered subcutaneously to the respective groups. After 30 min of vehicle/test drugs administration, nor-adrenaline (0.1 mg/kg, i.v.) or adrenaline (0.05 mg/kg, i.v.) or isoprenaline (20 µg/kg, i.v.) are injected. The pupil diameters of each animal is measured. The mean value in each group is reported.

Interpretation: Mean pupil diameter in test group is compared with control group. The sympatholytic drugs antagonize the mydriatic effects of sympathomimetics. Therefore, the pupil diameter of test groups is lesser than the control group (Freundt, 1965).

B. **Blood pressure response in dogs:**

Sympatholytics decreases the blood pressure by blocking α and β receptors. This model is also used to determine the specific receptor antagonizing property of test compound by using specific receptor agonist. Rats and cats are also used to monitor changes in blood pressure induced by drugs.

Method: Male Mongrel dogs (15–20 kg) are used in this model. Animals are anesthetized with chloralose (100 mg/kg, i.v.) or methohexital (10 mg/kg, i.v.), and maintained with nitrous oxide and oxygen at 4:2 proportion in a non-rebreathing system using a respiratory pump. Animals are immobilized by succinylcholine (1 mg/min). Brachiocephalic veins were cannulated, one for dextrose (5% w/v) infusion and the other for the succinylcholine infusion. Left carotid artery is cannulated and connected to a blood pressure recorder. The femoral vein is cannulated for drug administrations. Atropine is also injected (2 mg/kg) at the start of the experiment to avoid reflex vagal action. Phentolamine (1 mg/kg) or propranolol (1 mg/kg) is used as a standard sympatholytic drug. After recording the baseline, vehicle/standard/test drug is administered via the femoral vein and blood pressure is recorded. Blood pressure of standard and test drugs are compared with vehicle treatment (Olivares et al., 1967).

Interpretation:

Sympatholytics decrease the blood pressure as compared to vehicle. Specific sympatholytics like a blocker also block the response of α agonist like noradrenaline on blood pressure.

C. **Nictitating membrane prolapses in cats:**

The nictitating membrane is a transparent or translucent third eyelid present in some animals like cats and dogs that can be drawn across the eye for protection and to moisten it while maintaining vision. The cat is more suitable than a dog for the recording of nictitating membrane contractions because the former has a developed nictitating membrane and it is better responding to the stimulation of cervical sympathetic

trunk. Sympatholytics exert relaxing effect on this membrane.

Method:

Cats (3–5 kg) are used in this model. Animals are divided into control (vehicle) and test group (test sympatholytic). Vehicle/test sympatholytic is administered orally and after 30 min, degree and duration of the prolapse of the nictitating membrane are scored. The relative affinity of the test compound is compared by mean duration of prolapse in hours by the dose in mg/kg body weight.

Interpretation: Sympatholytic agents relax the nictitating membrane and the results are compared with the control group (Short et al., 1963; Turner, 1965).

IN VITRO MODELS

A. **Isolated frog heart preparation:**

Isolated heart preparation is also used to evaluate the sympatholytic activity on the heart. The sympatholytic like propranolol reduces the heart rate by blocking of β receptor or membrane stabilizing effect on the heart.

Method:

The method is similar as described in the previous chapter (Animal models for screening sympathomimetics). In this model, sympatholytics are injected instead of sympathomimetics. After recording baseline (basal heart rate), propranolol (200 μg/ml)/test Sympatholytics are administered and record the response by using force transducer on physiograph. Observed heart rate is compared with basal heart rate (Bagayath and Kothapalli, 2014; Smith, 1952). Similarly, effect of sympatholytics on sympathomimetics induced changes in the heart rate can also be evaluated.

Interpretation: Sympatholytics reduce the basal or sympathomimetics induce increase in heart rate.

B. **Isolated aortic muscle strips:**

This model is used for evaluation of both sympathomimetics and sympatholytic agents. Aortic muscles contain α_1 receptor. Sympathetic stimulation contracts the aorta while blocking of sympathetic α_1 receptor relax the aortic muscle. In this model, rabbit, cat, and rat are generally used to isolate the aortic muscle.

Method:

Cat (3-5 kg), rabbit (2-3 kg) or rat (150-200 g) are dissected to expose thoracic aorta. The thoracic aorta is isolated and kept in Krebs solution with aeration. Extra fats and connecting tissues are removed. Two

spiral aortic strips (20–30 mm length) are cut from thoracic aorta. The spiral aorta is mounted separately in organ bath containing Krebs solution. One is used as a standard and other is used for the test. The tissue preparations are allowed to stabilize for 60–90 min. During the equilibration period the bathing solution is replaced with a fresh solution in every 15–20 min. The strips were connected to isotonic lever of the gravity type adjusted to give 5-fold magnification and counterweighted to exert 0.5–1 g resting tension. The recording was taken on kymograph. Percentage inhibition of standard (e.g. phenoxybenzamine) and test drug are recorded against noradrenaline-induced contraction.

Firstly noradrenaline is added in cumulative doses (2×10^{-8} to 10^{-4} M) without changing the bath solution. Doses were given at 5 min intervals. Cumulative response curve of noradrenaline is recorded. Then tissue preparations are washed three times with fresh Krebs solution. After proper washing standard phenoxybenzamine 1×10^{-6} M or test preparation are added and left in the bath for 10 min. Noradrenaline is then added in the same series and observes the inhibition response. IC_{50} value of test drug can also be calculated by using different doses. The α_1 receptor blockers decrease the contractile response of noradrenaline (Ghose, 2008; Hoshino and Fujieda, 1980; Olivares et al., 1967).

C. **Isolated guinea pig tracheal chain:**

This model is used to determine β-agonistic and antagonistic activity. Stimulation of β_2 relaxes the tracheal muscle which is inhibited by β_2 antagonist. To evaluate the β_2 sympatholytic activity, percent inhibition of isoprenaline induced relaxation is observed after test drug administration and that is compared to maximum relaxation response of isoprenaline alone. The guinea pig tracheal chain is more sensitive than rats and other animals thus guinea pig is generally used in this test.

Method:

Albino guinea pig (300–350 g) is sacrificed and the tracheal chain is isolated. The trachea is then cut into 6–10 rings of the same width and connected in series by a silk thread. The rings are mounted on an organ bath containing Tyrode solution, aerated with carbogen mixture, and maintained at 37°C. A weight of 0.5 g tension is applied on the writing lever. After stabilization of tissue preparation, isoprenaline (10^{-8} mM to 10^{-3} mM) is added in cumulative doses to obtain a maximal relaxing response on kymograph. Thereafter, tissue is washed out at least 3–5 times. Test compound is then added in a bath and after 5–10 min, isoprenaline is again added in cumulative doses and response is recorded. Tissue is washed again and repeated with increased test dose. Percentage inhibition response is calculated in respect of isoprenaline alone. IC_{50} value can also be calculated.

Interpretation: β_2 sympatholytic activity is considered if the test drug inhibits the relaxing response of isoprenaline

PARASYMPATHOMIMETICS

Parasympathomimetic drugs are also known as cholinomimetic/cholinergic drugs. Acetylcholine is an endogenous neurotransmitter, which acts at cholinergic synapses and neuroeffector junction in the central and peripheral nervous system. Parasympathomimetic drugs stimulate the action of the parasympathetic nervous system by activating the cholinergic receptors (muscarinic M and/or nicotinic N receptor) and/or increasing the availability of acetylcholine. Muscarinic receptor mainly present in the gastric glands (M_1 receptor), heart (M_2 receptor), and smooth muscles (M_3 receptor) and their activation causes the gastric secretion, cardiac depression, and smooth muscle contraction. Nicotinic receptors are present in the autonomic ganglion (N_n receptor) and neuromuscular junction (N_m receptor) and their activation cause the stimulation of autonomic ganglion and skeletal muscles (Gupta, 2008).

21.2 MODELS FOR SCREENING PARASYMPATHOMIMETICS

The models for screening parasympathomimetics are based on the action of standard acetylcholine on the particular parasympathetic innervations. Cardiac depression, smooth muscle contractions, and eyes accommodation, are the determinant effect of parasympathomimetics.

Table: Animal Models Used for Screening Parasympathomimetics

Sr. No.	Screening Models	Description
I	**In Vivo Models**	
A	Blood pressure response in anesthetized animals	Dogs, cats, and rats are generally used in this model. Intravenous (femoral vein) injection of acetylcholine (2 µg) produces a fall in blood pressure due to vasodilatation.
B	Miosis effect on rabbit eye	Parasympathomimetic agents produce miosis (pupil contraction) in the eye by contracting the ciliary muscle. In rabbits, test drugs are applied topically, while in rodents they are injected intraperitoneally. The pupil diameter is observed and recorded.
II	**In Vitro Models**	
A	Isolated heart	This model is used to assay parasympathomimetic drugs by monitoring heart rate.

Sr. No.	Screening Models	Description
	preparation	Parasympathomimetics such as acetylcholine suppress the pacemaker activity, resulting in a decrease in heart rate.
B	Isolated ileum preparation	The intestinal ileum of guinea pigs, rats, and rabbits is used in this model. GI smooth muscle contains **M_3 receptors**, which cause contraction during parasympathetic stimulation. Bioassay techniques are used to determine the potency of the test substance by comparison with a standard acetylcholine solution.
C	Frog's rectus abdominis muscle preparation	The frog's rectus abdominis muscle is used to assay spasmolytic drugs. Stimulation of the parasympathetic system produces contraction of the muscle preparation.
D	Isolated tracheal chain preparation	Similar to GI smooth muscle, parasympathetic stimulation causes contraction of the tracheal chain. Guinea pigs are commonly used to isolate the tracheal chain for this preparation.

IN VIVO MODELS

A. **Blood pressure response in anesthetized animals:**

Parasympathetic stimulation produces fall in blood pressure due to vasodilatation. The acetylcholine (2 µg) is used as a standard. Blood pressure response depends upon the dose of acetylcholine. Acetylcholine at 2 µg causes fall in blood pressure without any changes in heart rate, while at high dose (10–50 µg) produces fall in blood pressure along with bradycardia due to cardio-inhibition. The large dose in the range of 2–5 mg causes increase in blood pressure and heart rate, both due to nicotinic action on the sympathetic ganglia. In this model, dogs and cats can be used. This model is used to evaluate the cholinomimetic and anticholinesterase activity.

Method: Animals are anesthetized with a suitable anesthetic agent and prepared for blood pressure monitoring. Animals are kept on artificial respiration. The carotid artery is cannulated for recording blood pressure, the femoral vein is cannulated for injecting the test drug. After administration acetylcholine is a standard drug is injected and blood pressure is recorded.

Interpretation: The fall in blood pressure after administration is compared with the fall produced by the standard acetylcholine.

2. **Miotic effect on rabbit eyes**

Parasympathomimetics like carbachol, pilocarpine, and physostigmine contract the pupil (miosis) and decrease the intraocular pressure by muscarinic (M_3 receptor) action. Parasympathetic system modulates the eye accommodation through the control of circular muscles in the eye. The activation of M_3 muscarinic receptors causes ciliary muscle contraction, resulting in decrease in diameter of the ring of ciliary muscle. Whereas parasympatholytics (e.g., atropine) oppose the miosis effect, it dilates the pupil (mydriasis). This model is used for the evaluation of both parasympathomimetic and parasympatholytic agents.

Method: Male rabbits (2–3 kg) are generally used in this model. Animals are divided into different groups: control (vehicle treatment), standard, and test group (test sympathomimetic treatment). 0.5–2% pilocarpine and/or 0.25–0.5% physostigmine ophthalmic solution is used as a standard. Eyes of rabbits are washed with distilled water and left for 5 min. Vehicle/standard/test drug is applied topically to their respective groups and measured the pupil diameter. Calculate the mean pupil diameter in each group.

This method can also be performed on rats (150–200 g) and mice (25–30 g). Vehicle/test drug is injected intraperitoneally. After 30 min pupil diameter is monitored at every 10 min interval upto 120 min.

Interpretation: Mean pupil diameter of standard and test groups are compared with the control group. The topical administration of standard or other parasympathomimetics decrease the pupil diameter and produce miotic effect (de Elio, 1948; Edwards et al., 1960).

IN VITRO MODELS

A. **Isolated heart preparation:**

Isolated heart preparation model is also used for evaluation of parasympathetic system similar to the sympathetic system. Parasympathomimetics like acetylcholine (2 µg) decrease the force of contraction and heart rate by activation of M_2 receptor on heart, while parasympatholytic (atropine 20 µg) administration has little effect on the heart but opposes the effect of parasympathetic stimulation. In this model, frog's or rabbit's heart is used. The detailed methodology is similar as described in chapter Animal models for screening sympathomimetics (Broadbent, 1963).

B. **Isolated ileum preparation:**

Gastro-intestinal ileum preparation model is the most commonly used model for evaluating of parasympathomimetics agents. GI smooth muscles contain muscarinic M_3 receptors, which are activated by parasympathetic stimulation. Activation of M_3 receptor causes contraction of GI smooth muscle. Whereas blocking the M_3 receptor by its antagonists (e.g., atropine, hyoscyamine, etc.) oppose this effect.

Guinea pigs, rats and mice are commonly used to isolate the ileum. For the evaluation of ganglion stimulants such as nicotine, rat's jejunum is used instead of the ileum.

Method: The animal is sacrificed and intestinal part is removed and kept in Tyrode solution. Ileum (2–3 cm in length) is then isolated and extra fatty tissue, nerve and blood vessels are removed. Then ileum is mounted on an organ bath containing Tyrode solution with carbogen aeration. The organ bath is maintained at 35–37 °C. The writing lever is tied up with tissue and extra 0.5 load tension is applied over the lever for proper tissue relaxation. After stabilization of tissue preparation, baseline is recorded on kymograph, then dose-response curve of standard acetylcholine solution (1 µg onward) is recorded upto ceiling effects. The tissue is washed with fresh Tyrode solution 3–4 times at 1 min interval of each response. Then a test preparation (agonist) is added and contraction response is recorded. The response of test is matched with the standard by adding acetylcholine.

Interpretation: The potency of an agonist is determined by comparing its contraction with known standard preparation. If the contraction is observed after adding test substance, similar to standard substance, it is considered as a parasympathomimetic activity (agonist). If contraction does not occur after adding test substance and the addition of acetylcholine produces decreased response in the presence of test, it is considered as an antagonist of acetylcholine receptor (parasympatholytic) (Ghose, 2008; Gupta, 2008; Peddi Reddy, 2011).

C. **Frog's rectus abdominis muscle preparation:**

Rectus abdominis muscle is a voluntary muscle preparation, which produces slow contraction in response to acetylcholine. This preparation is suitable for the assay of parasympathomimetic and parasympatholytic drugs. It is also used for assay curare like substances (spasmolytics).

Method: Frog is sacrificed and rectus muscle is isolated. Isolated rectus is then ligated and mounted in an organ bath containing Frog Ringer solution. The simple or Gimbal lever is used to record the contraction response on a kymograph. After stabilization, acetylcholine standard preparation (1 µg onwards) is added to record the response upto ceiling response. Tissue preparation is washed out after each response (2–3 times at 1 min interval). Then a test preparation (agonist) is added and contraction response is recorded. The response of test is matched with the standard by adding acetylcholine to find out the potency of the test.

For evaluation of parasympathetic and spasmolytic agents, dose-response curve of acetylcholine is recorded. Then tissue is washed out and the test substance is added in a bath solution and after 90 sec

without washing known standard acetylcholine solution is added and record the response. Parasympatholytic and spasmolytic test drugs decrease the contraction response of acetylcholine (Jde Elio, 1948; Ghosh, 2008). However, this method is absolute due to ethical code.

D. Isolated tracheal chain preparations:

In this method guinea pig tracheal chain is prepared and mounted on a student organ bath with aeration and is maintained at 35 to 37 °C in a physiological salt solution (Krebs solution). The response of test or standard drugs is recorded as described in the isolated tissue preparation method.

PARASYMPATHOLYTICS

Parasympatholytics or anticholinergic drugs reduce the activity of parasympathetic nervous system and antagonize the action of acetylcholine by blocking cholinergic receptors. The blockade of muscarinic receptors abolish the action of acetylcholine and produce spasmolytic activity on bronchial and GI smooth muscles, pupil dilation, inhibit gastric secretions and increase the heart rate. Moreover, antagonism of nicotinic receptor produces ganglionic inhibition and relaxes the skeletal muscle. Nicotinic receptor blockers are also known as ganglion (N_N) blockers and neuromuscular junction (N_M) blockers (Brunton et al., 2006).

22.2 MODELS FOR SCREENING PARASYMPATHOLYTICS

The models for screening parasympatholytics are similar as models of parasympathomimetics. The parasympatholytics oppose/block the effects of parasympathomimetic like acetylcholine. The several models are discussed in the previous chapter (parasympathomimetics); the important models applicable to this topic are discussed in the chapter.

Table: Animal Models Used for Screening Parasympatholytics

Sr. No.	Screening Models	Description
I	**In Vivo Models**	
A	Blood pressure response in dogs	Dogs are generally used in this model. Intravenous (femoral vein) injection of atropine (mg/kg), a parasympatholytic, increases heart rate and blocks the blood pressure and heart rate responses produced by acetylcholine.
B	Mydriasis effect in eye	Parasympatholytics produce mydriasis (pupil dilation) in the eye. In rabbits, test drugs are applied topically, while in rodents they are injected intraperitoneally. The change in pupil diameter is observed.
C	Acetylcholine-induced bronchoconstriction in guinea pigs	Guinea pigs are exposed to acetylcholine (10% aerosol) in a histamine chamber, which causes bronchial smooth muscle contraction leading to preconvulsive dyspnoea. Pretreatment with parasympatholytic drugs delays the onset of these symptoms.

Sr. No.	Screening Models	Description
II	**In Vitro Models**	
A	Isolated ileum preparation	The intestinal ileum of guinea pigs, rats, and rabbits is used. Parasympatholytics inhibit the contractile response produced by acetylcholine by blocking **M_3 receptors** on gastrointestinal smooth muscle.
B	Frog's rectus abdominis muscle preparation	The frog's rectus abdominis muscle is used to assay spasmolytic and parasympatholytic drugs. Parasympatholytics inhibit the contraction produced by acetylcholine.
C	Isolated heart preparation	Parasympatholytics such as atropine increase heart rate by blocking the action of acetylcholine on the heart.

IN VIVO MODELS

A. **Blood pressure response in dogs:**

Parasympatholytic like atropine decreases the heart rate and it blocks the blood pressure and heart rate response of acetylcholine.

Method: Dogs are anesthetized with a suitable anesthetic agent and prepared for blood pressure and heart rate monitoring. Animals are kept in artificial respiration. The carotid artery is cannulated for recording blood pressure. The femoral vein is cannulated for injecting atropine/acetylcholine/test parasympatholytic drugs. After stabilization different doses of acetylcholine (2–10 µg/kg) are injected and recorded the blood pressure and heart rate. A single dose of atropine standard (1 mg/kg)/test drug is administered and the responses are monitored. Then again acetylcholine is injected in the same doses to record the blood pressure and heart rate.

Interpretation: The response of acetylcholine before and after parasympatholytics is compared. The parasympatholytic agents block the responses of acetylcholine to increase the heart rate (Broadbent, 1963).

B. **Mydriasis effect in eye:**

Parasympatholytics like atropine and tropicamide dilate the pupil by blocking the muscarinic (M) receptor action on the eye.

Method: Rabbits (2–3 kg), rats (150–200 g) and mice (25–30 g) are generally used in this model. Animals are divided into different groups: control (vehicle treatment), standard, and test.

In rabbits, vehicle/standard (0.1 % w/v atropine)/test drugs are applied topically and in rodents, drugs solution are injected intraperitoneally. Five min after topical application and 30 min after intraperitoneally injection, pupil diameters of each animal is monitored. Mean pupil diameter of each group is calculated.

Interpretation: Mean pupil diameter of standard and test groups are compared with the control group. The administration of standard or other parasympatholytics increase the pupil diameter and produce mydriasis effect in the eye (de Elio, 1948; Edwards et al., 1960).

C. **Acetylcholine-induced bronchoconstriction in guinea pigs:**

Acetylcholine causes the bronchi smooth muscle contraction resulting in hypoxia and finally leads to convulsion. Histamine chamber (Fig. 22.1) is used in this method. Acetylcholine (10%) is to be given as aerosol under a constant pressure 40 mm/Hg from the inbuilt nebulizer of the histamine chamber. The time required for appearance of preconvulsive dyspnoea symptoms are monitored. Parasympatholytics can delay the occurrence of these symptoms.

Method: Guinea pigs are generally used in this model. Animals are divided into control and test group. Each animal is placed in histamine chamber before the treatment and exposed to acetylcholine (10%) aerosol and the time required for appearance of preconvulsive dyspnoea is recorded. After that animals are removed from the chamber and left into the fresh air for 24 hours. Next day animals from the control group are treated with vehicle and animals of test group are treated with the test parasympatholytic. Thirty min after the treatment, animals are placed again in histamine chamber with acetylcholine (10% aerosol) and the time for preconvulsive dyspnoea is recorded. Percent increase of preconvulsive time is calculated.

Interpretation: Results of test group are compared with control group. If the test drug delays the preconvulsive dyspnoea time, the drug is considered as a parasympatholytic agent (Tripathi and Dias, 1977; Patil and Ninawe, 2016).

A. **Isolated ileum preparation:**

Similar to the assay of parasympathomimetics, gastrointestinal ileum preparation is also a most common model for evaluating of parasympatholytic agents. Parasympatholytics block the contractile response of parasympathetic stimulation. The guinea pigs, rats, rabbits, and cock is used to isolate the ileum. The percentage inhibition of a parasympatholytic is recorded against acetylcholine-induced contraction.

Method: The method of tissue preparation is same as described in Chapter 21 (parasympathomimetics). After stabilization of tissue preparation baseline is recorded on kymograph, then dose response curve of standard acetylcholine solution (1 µg onward) is recorded upto ceiling effect. The tissue is washed with a fresh Tyrode solution (2-3 times). Then a standard atropine (3.5 nM or 0.01 µg/ml concentration in an organ bath)/test preparation (antagonist) is added in organ bath for 2-5 min and without washing standard

acetylcholine solution is added to record the response again with the same dose. Calculate the percentage inhibition response.

In this method, cumulative antagonism can also be determined by recording cumulative dose-response curve of an agonist (acetylcholine) with and without the presence of an antagonist. By using different concentrations of an antagonist, K_{50} value can be calculated (Ghose, 2008; Iean et al., 2012).

B. **Frog's rectus abdominis muscle preparation:**

This model is employed to assay the parasympatholytic activity. Parasympathetic stimulation contracts the rectus abdominis muscle preparation, which is abolished by parasympatholytics. Frog's rectus abdominis muscle is used in this model.

Method: This model is described in the previous chapter 21 parasympathomimetics. After stabilization of tissue preparation cumulative dose response curve of acetylcholine is recorded with and without the presence of an antagonist. After recording the cumulative dose-response curve of an agonist, the tissue is washed with fresh Tyrode solution (2-3 times). Then test parasympatholytic is added in organ bath solution and after 1-2 min without washing again cumulative dose response curve of acetylcholine is recorded.

Interpretation: If percentage inhibitory response is observed after adding the test drug, it is considered as a parasympatholytic. By using different concentrations of an antagonist the pA_2 value can also be calculated (Schild, 1947; Ghose, 2008).

SKELETAL MUSCLE RELAXANTS

The muscle relaxants are the drugs that decrease the skeletal muscle tone. The skeletal muscle relaxants are generally used to relieve muscle spasms, pain, and hyper reflexia. The muscle relaxants are categorised into two therapeutic groups: neuromuscular blockers and spasmolytics. Neuromuscular blockers act on the periphery by blocking the neurotransmission (acetylcholine) at neuromuscular end plate (nicotinic acetylcholine receptors, Nm). Neuromuscular blocking agents are distinguished by whether or not they cause depolarization of the motor end plate. Neuromuscular blockers are classified into two groups (a) competitive (stabilizing) agents like d-tubocurarine and (b) depolarizing agents such as succinylcholine, decamethonium. The spasmolytics, also known as “centrally acting” muscle relaxants and are used to relieve musculoskeletal pain and spasms.

In the screening of neuromuscular blocking agents, the following parameters should be considered: potency of the drug, the time required for maximal effect, duration of action, cumulative effects and

reversibility by antagonism. Neuromuscular blockers may show different effects in in vivo models and in an in-vitro model. Therefore, different tests should be applied for assessing one agent (Gupta, 2004).

23.2 MODELS FOR SCREENING SKELETAL MUSCLE RELAXANTS

Skeletal muscle relaxants or neuromuscular blocking agents decrease the skeletal muscle tone by inhibiting the parasympathetic action on the neuromuscular end plate. Several in vivo and in vitro models (Table 23.1) are available for screening the skeletal muscle relaxants

Table: Animal Models Used for Screening Skeletal Muscle Relaxants

Sr. No.	Screening Models	Description
I	**In Vivo Models**	
A	Rotarod test for skeletal muscle relaxant activity	Rodents are used in this model. Administration of centrally acting muscle relaxants such as diazepam or d-tubocurarine decreases muscular tone and reduces the fall time on a rotating rod (rotarod).
B	Activity reduction test	Skeletal muscle relaxants relax skeletal muscles and reduce motor activity. Albino mice or rats are generally used in this test to observe reduction in spontaneous activity.
C	Rabbit head-drop model	Intravenous injection of skeletal muscle relaxants causes head drop and delay in raising the head. This method determines the minimal intravenous dose required to produce head drop. These drugs also decrease gripping strength.
D	Inclined screen and inverted grid method	Animals are placed on an inclined screen or inverted grid. Muscle relaxants reduce the ability of animals to hold onto the surface, indicating reduced muscle strength and coordination.
E	Sciatic nerve–gastrocnemius muscle preparation in rabbit	In anesthetized rabbits, the sciatic nerve is ligated and stimulated through electrodes. After administration of muscle relaxants, the contractile force of the gastrocnemius muscle decreases.
II	**In Vitro Models**	
A	Frog's rectus abdominis muscle preparation	The frog's rectus abdominis muscle is used for assay of spasmolytic drugs. Skeletal muscle relaxants inhibit acetylcholine-induced muscle contraction.
B	Isolated nerve–muscle preparation	Amphibian or mammalian nerve–muscle preparations are isolated and immersed in Krebs solution. Electrical stimulation through electrodes induces contraction, which is inhibited by neuromuscular blocking agents.
C	Phrenic nerve–diaphragm preparation	Rats and guinea pigs are used to isolate respiratory muscles in this preparation. Electrical stimulation of the phrenic nerve produces contraction, which is inhibited by neuromuscular blocking agents that block neuromuscular transmission.

IN VIVO MODELS

A. **Skeletal muscle relaxant activity by using rotarod**

Skinner and Young (1947) firstly evaluated the muscle relaxant property using the rotating cylinder in mice.

Rotarod apparatus:

The rotarod apparatus is useful for screening of drugs that affecting motor coordination.

It consists of rotating rod of metal, 3 cm diameter, and having speeds of approx. 5 to 25 revolutions/min. Skeletal muscle relaxant drugs decrease the muscle gripping strength of animals and decreases fall down time on the rotarod. Method: Albino mice (25–30 g) are used in this model. Animals are divided into three groups, each group contains 5–6 animals. Control group is treated with vehicle; the standard group treated with diazepam (10 mg/kg p.o. or 3–5 mg/kg i.p.) and test group received test drug. Thirty min after oral administration of drugs, animal is placed on rotating drum (speed : 25 rpm) and recorded the fall time of each animal. The mean fall time of each group is calculated and compared with the control group.

nterpretation: Skeletal muscle relaxant drug decreases the mean fall time as compared to the control group (Al-Naggar et al., 2003).

B. **Skeletal muscle relaxant activity using actophotometer:**

Similar to the CNS depressant drug, skeletal muscle relaxant is also assessed by locomotor activity. Skeletal muscle relaxants relax the muscle and lead to decrease the locomotion. The locomotor activity is assessed with the help of actophotometer, which consists of photocells in the outer wall. Interruptions of photocell beams (locomotor activity) are recorded by means of a six-digit counter. Rodents are used in this model.

Method: Albino mice (25–30 g) or rats (150–200 g) are selected and divided into control and test group. Control rats receive vehicle and test groups receive test drugs. After 30 min of oral administration, animals are subjected to locomotion count. Each animal is placed in the actophotometer and locomotion count for 10–15 min is recorded. The mean locomotion count is calculated and compared with the control group.

Interpretation: If the test drug decreases the locomotion count as compared to control, the test drug is considered as a muscle relaxant (Tirumalasetty et al., 2012; Vogel, 2002). However, CNS depressant drugs also reduce locomotion without muscle relaxation.

C. **Rabbit head-drop model:**

The rabbit head-drop method was firstly described by Varney et al., 1948–1949. This method is used to measure the minimal intravenous dose of the drug required to produce a head drop. Rabbits are commonly used in this model. Instead of rabbit, mouse, rat, guinea pig, dog, and monkey can also used in this method.

Method: Rabbits (2–3 kg) are divided into standard and test group, each group contains 5–6 animals. The standard group is treated with d-Tubocurarine hydrochloride (0.012% w/v in saline) and test group is

treated with test preparation. Animals are hold and standard/test drug is injected (0.1 ml/15 sec or 0.4 ml/min) into the marginal vein of a rabbit's ear till the rabbit's neck muscles are relaxed such that the animal cannot hold its head up. The total amount of test sample required to produce the endpoint is compared with the total amount of the standard sample required to produce similar endpoint. ED_{50} value is also calculated.

D. **Inclined screen and inverted grid method:**

The inclined screen at 50° from horizontal is used in this model. Animals with skeletal muscle relaxation abruptly sliding off the screen. This model is used to assess the ability to stay on an inverted grid after administration of neuromuscular blocking agents.

Method: Albino rats (150–200 g) or mice (25–30 g) are used in this model. Animals are divided into control and test group. Control group receives vehicle and test group receives test drug. 30 min after drug administration, animals are placed on inclined screen (50°) and the fall time is recorded. Mean fall time is calculated and compared with the control group. By using different doses of test drug, the ED_{50} value can also be calculated (Gupta 2004).

E. **Sciatic nerve-gastrocnemius muscle preparation in the rabbit:**

Levis et al (1953) were first described the sciatic nerve-gastrocnemius muscle preparation in the rabbit as a model for testing neuromuscular blocking agents. Skeletal muscle relaxant drug attenuates the contraction against stimulator.

Method: Rabbits (1–2 kg) are used in this test. Animals are anesthetized and ligated the sciatic nerve. Then the nerve is cut and a shielded electrode is placed on the peripheral portion of the nerve. A thread is attached to the tendon of the muscle. The force transducer is attached to the recorded force of contraction. The twitches of the muscle are elicited by supramaximal stimulation (250 cps, 1 ms, at 15 V applied for 0.2 sec in every 10 sec). Vehicle/test drugs are administered intravenously into the marginal ear vein. The force of contraction after test drug is compared to the value of control (vehicle) group.

Interpretation: If the test drug decreases the force of contraction against stimulation.

IN VITRO MODELS

A. Frog's rectus abdominis muscle preparation:

This model is used to assay the skeletal muscle relaxant drugs. Test drugs inhibit the acetylcholine-induced

muscle contraction. Frog's rectus abdominis muscle is used in this model.

Method: This model is described in the previous chapter parasympathomimetics. After stabilization of tissue preparation, cumulative dose response curve of acetylcholine is recorded with and without the presence of skeletal muscle relaxant. After getting the cumulative dose response curve of acetylcholine, the tissue is washed out 2–3 times and then test drug is added in organ bath medium. After 1–2 min without washing again cumulative dose response curve of acetylcholine is recorded.

Interpretations: Percentage inhibitory response is observed after adding the test drug.

It is considered as a skeletal muscle relaxant. By using a different concentration of an antagonist, the IC_{50} value can also be calculated

DRUGS ACTING ON EYES

The eye is a complex sensory organ responsible for vision. Injury or disease to the ocular system can result in vision loss. A variety of ophthalmic preparations (topical, parenteral, and oral) are available for both therapeutic and diagnostic use. Medications play a key role in the management of eye accommodation, diabetic retinopathy, glaucoma, infection, inflammation, and macular degeneration. In this chapter, we will focus on animal models of glaucoma, cataract, and retinopathy.

ANIMAL MODELS OF OCULAR PUPIL ACCOMMODATION

The pupil dilation and contraction are controlled by the iris muscle fibers (circular and radial) that are triggered by parasympathetic and sympathetic system, respectively. The rabbit eye model is the most common model to assess the pupil dilation and contraction by topical administration of parasympathetic and sympathetic drugs. The stimulation of sympathetic and parasympathetic nerves produces mydriasis and miosis respectively and their blocking produces opposite effects. The detail description of this model is discussed in previous chapters 21 and 22 (Sympathomimetics and Parasympathomimetics).

ANIMAL MODELS FOR LOCAL OCULAR ANESTHESIA

Topical administration of local anesthesia on eye reduces the corneal reflexes and produces miosis. The retrobulbar block in dogs and surface anesthesia on the cornea of rabbits are common models to assess the local anesthetic effects. The detail description of these models is described in Chapter, Local Anesthetics.

ANIMAL MODELS FOR GLAUCOMA

Glaucoma is an ocular disease that is characterized by high intraocular pressure (IOP) and further leads to ocular blindness. The rise in IOP is caused by increasing aqueous formation and reducing outflow. The increased IOP may trigger retinal ganglion cells and optic nerve damage. The normal IOP in human being ranges from 10–20 mmHg and can rise upto 60–70 mmHg in glaucoma patients. Several drugs are used for treatment of glaucoma like topical preparation of pilocarpine, physostigmine, epinephrine etc and systemic preparation of acetazolamide mainly. ACE inhibitors etc. A wide variety of animal models has been used to study glaucoma. These include monkeys, dogs, cats, rodents, and several other species.

Table Animal Models for Screening Anti-Glaucoma Drugs

Sr. No.	Type of Model	Screening Model
1	In vivo models	Steroid-induced glaucoma
2	In vivo models	Methyl cellulose-induced glaucoma
3	In vivo models	Hyaluronic acid-induced glaucoma
4	In vivo models	α-Chymotrypsin-induced glaucoma in rabbit
5	In vivo models	Autologous ghost RBC-induced glaucoma
6	In vivo models	Water-loaded rabbit model of glaucoma
7	In vivo models	Laser-induced glaucoma

1. **In Vivo Models:**

A. **Steroid-induced glaucoma**:

Administration of glucocorticosteroids can lead to the development of ocular hypertension and glaucoma through a reduction in aqueous humor outflow. Models using steroid-induced ocular hypertension have been developed in many animals such as rabbits, bovine, and sheep. A topical application of prednisolone acetate (1% w/v) induced IOP in bovine and sheep (from 16-17 mmHg to 30-35 mmHg and from 11.2 mmHg into 23.2 mmHg in bovine and sheep, respectively). IOP in these animals returned to normal when the treatment was discontinued. In rabbits, injection of betamethasone subconjunctivally or α-chymotrypsin into the posterior chamber also reported to elevate IOP that lasted for 7 weeks. The consistency and robustness of the IOP response developed using steroids and the low cost of maintaining the animals (rats, rabbit, sheep and cows) compared to primates are important advantages of this model. However, the prolonged topical corticosteroid treatment required to achieve glaucoma can cause significant adverse effects such as cataracts and corneal ulcer (Armaly 1971; Jones and Rhee 2006).

Method: Adult rabbits or cats are used in this test. Animals are divided into three groups, each containing 5-6 animals. The groups are divided into normal (vehicle control [betamethasone]), and test group (dexamethasone + test drug). The normal group is treated with sterile saline topically in control group and test groups, glaucoma is induced by 10 µl topical administration of dexamethasone (0.5-1% w/v) 2-3 times per day in the eye for 2-3 weeks. The test group is treated with test preparation along with dexamethasone. The IOP is monitored weekly by using Schiotz tonometer (Fig. 24.1) or tonopen.

Interpretation: The mean IOP values are calculated. The mean values of the control group are compared with normal values to determine the induction of glaucoma (IOP) and test values are compared with control to determine the anti-glaucoma effect of the test drug. Anti-glaucoma drug reduces the mean IOP as compared to control group (Jones and Rhee, 2006).

B. **Methyl cellulose-induced glaucoma:**

Local injection of methyl cellulose (0.5-1% w/v) in the anterior chamber of the eye can increase the IOP and lead to glaucoma. Initially it can rise IOP from 20 mmHg to 30 mmHg in next 2 h then it produces consistent intraocular hypertension within 6-8 weeks.

Method: Rabbits are used in this test. Animals are divided into three groups each containing 5-6 animals. The groups are divided into normal (saline), control (methyl cellulose), and test group (methyl cellulose + test drug). The normal group is treated with sterile saline locally. In control group and test group, glaucoma is induced by a series of four intra-anterior chamber injection of methyl cellulose (100-200 µl 0.5-1% w/v) in the eye. The intraocular hypertension is produced within 6-8 weeks. The test group is treated with test preparation along with methyl cellulose. The IOP is monitored weekly by using tonometer (tonopen).

Interpretation: The mean IOP values are calculated. The results of the test group are compared with the control group. If the test drug attenuates the IOP against methyl cellulose it is considered as an antiglaucoma drug.

C. **Hyaluronic acid-induced glaucoma:**

Rats are used in this model. Hyaluronic acid is injected weekly in the rat anterior chamber of one eye whereas the contralateral eye is injected with saline solution. Using a Hamilton syringe with a 30-gauge needle, 25 µl of hyaluronic acid in sterile saline solution is injected weekly into one eye of anesthetized rats while an equal volume of vehicle saline solution is injected in the fellow control eye eye and IOP are

recorded weekly by using ophthalmoscope and tonometer (Tonopen). IOP of the control eye is compared with the normal eye. Hyaluronic acid administration increase the IOP as compared to normal (Moreno et al., 2005).

SALT SCREENING MODEL FOR CATARACT

Cataractous is one of the leading causes of visual dysfunction and contributes to 50% of blindness worldwide. Cataract is characterized by the clouding or opacity of the eye lens. Progression of cataract depends on the several risk factors such as aging, UV-radiation, smoking, diabetes and systemic hypertension. The lenticular oxidative stress is the key factor for cataractogenesis. The enhance oxidative stress may further lead to oxidative damage of lens protein and ion pumps resulted in protein aggregation and ionic imbalance that exacerbate the cataract formation. The lens opacity in-vitro and in-vivo is the characteristic measurement parameter of assessment of cataract formation. Apart from lens opacity, lens antioxidants like superoxide dismutase, catalase, glutathione, glutathione peroxidase, lipid peroxidation level (malondialdehyde) and lens ionic content (Ca^{2+} and Na^{+}) are also a measurable parameter. In cataract, lens opacity, ionic content, and malondialdehyde (the end product of lipid peroxidation) are increased and lens antioxidants or proteins are decreased. The lenticular opacity is monitored in-vivo by using slit lamp microscopy method and biochemical parameter is measured by the specific method.

Sr. No.	Type of Model	Screening Model
1	In vivo models	Selenite-induced cataract
2	In vivo models	Naphthalene-induced cataract
3	In vivo models	UV radiation-induced cataract
4	In vivo models	Diabetes-induced cataract
5	In vivo models	Cadmium chloride-induced cataract
6	In vivo models	Smoke-induced cataract
7	In vivo models	Microwave-induced cataract
8	In vivo models	Steroid-induced animal model
9	In vitro models	Isolated lens model
10	In vitro models	H_2O_2-induced model
11	In vitro models	Glucose-induced model

Sr. No.	Type of Model	Screening Model
12	In vitro models	Calcium-induced model

In Vivo Models

A. **Selenite-induced cataract:**

Sodium selenite induced cataract is the most common model that involves oxidative stress induced cataract. Sodium selenite causes oxidative damages of the lenticular cell and further leads to cataract formation.Method: Albino rats of either sex are generally used. Animals are divided into three groups each containing 5-6 animals. The groups are divided into normal, control, and test group. A normal group is treated with saline (10 ml/kg, p.o.). Control group is treated with single dose sodium selenite (19–30 μM/kg, s.c.). Sodium selenite gave to the suckling rats of 10–12 days of age, definitely before the completion of the critical maturation period of the lens at approximately 16 days of age. The test group is treated for 4–6 days after treatment of sodium selenite. After 16 days the opacity of the lens observed by using ophthalmoscope/slit-lamp microscopy during the protocol. Antioxidants, total protein, ionic contents, ATPase enzymes and lipid peroxidation were measured after the completion of the protocol. Anticataract test drug reduces the lens opacity and oxidative damages (Shearer et al., 1997).

B. **Naphthalene-Induced cataract:**

Naphthalene induced cataract resembles to human age-related cataract. The opacity of the lens is due to the formation of 1,2-dihydroxynaphthalene, a metabolite of naphthalene. This compound auto-oxidizes to 1,2-naphthoquinone, an agent that reacts with many metabolically active components of lens.

Method: Pigmented rats or albino rats are generally used. Animals are divided into three groups each containing 5–6 animals. The groups are divided into normal, control, and test group. A normal group is treated with saline or corn oil (10 ml/kg, p.o.). Control group is treated with naphthalene (10 % w/v in corn oil, 700–1000 mg/kg/day) for 2–3 weeks. The test group is treated with test drug 1 h prior to the naphthalene administration. Lens opacity is observed by using ophthalmoscope (Fig. 24.2) slit-lamp microscopy weekly during the protocol. Antioxidants, total protein, ionic contents, ATPase enzymes and lipid peroxidation were measured after the completion of the protocol. Anticataract test drug reduces the lens opacity and oxidative damages (Tao et al., 1991).

C**. UV radiation induced cataract:**

The lens is a major absorber of the UV radiation. UV radiation reaches the lens and generates free radicals, induces the oxidative stress and damages the lens proteins. Oxidative stress ultimately results in cataract formation.

Method: Albino rats are generally used for this study. The groups are divided into normal, control, and test group. A normal group is treated with saline (10 ml/kg, p.o.).

Control group is unilaterally exposed to UV-radiation (8 kJ/m²) in the 300 nm wavelength region for 1-2 weeks. The test group is treated with test drug along with UV exposure. Lens opacity is observed by using ophthalmoscope/slit-lamp microscopy weekly during the protocol. Antioxidants, total protein, ionic contents, ATPase enzymes and lipid peroxidation were measured after the completion of the protocol. Anticataract test drug reduces the lens opacity and oxidative damages (Ayala et al., 2000).

D. **Diabetes-induced cataract:**

The hyperglycemic condition causes the activation of the polyol pathway in the lens. The polyol pathway is also known as sorbitol-aldose reductase pathway. This is the major pathological pathway of sugar-induced and/or diabetic cataract. In diabetes condition, sugar is in high concentration in the aqueous humor of eyes and can diffuse passively into the lens. The enzyme aldose reductase, which is responsible for the conversion of glucose to sorbitol or galactose to galactitol via polyol pathway within the lens. These polyol is unable to diffuse passively out from the lens and thus produce osmotic gradient and causes the electrolyte imbalance in the lens and further induce the cataract formation. Diabetes or hyperglycemia is induced in albino rats by administration of STZ (35–65 mg/kg, i.p. single dose) or alloxan monohydrate (100–175 mg/kg, s.c. single dose); fructose solution (10 % w/v in drinking water for 10–12 weeks) or galactose solution (10–12.5 % w/v in drinking water for 5–6 weeks).

Method: Albino rats are generally used. Animals are divided into three groups each containing 5–6 animals. The groups are divided into normal, control, and test group. A normal group is treated with saline (10 ml/kg, p.o.). In control and test group, diabetes is induced by a suitable method. The test group is treated with test agent during the protocol. Lens opacity is observed by using ophthalmoscope/slit-lamp microscopy weekly during the protocol. Antioxidants, total protein, ion content, polyol content and lipid peroxidation are measured after the completion of the protocol. Anticataract test drug reduces the lens opacity and oxidative damages (Khan et al., 2016; Gupta 2004).

II. **<u>In Vitro Models:</u>**

A. **Isolated lens model:**

The isolated lens of pigs, goats, and rats are commonly used for the assessment of cataract formation. The cataract in the isolated lens is induced by several methods, like hydrogen peroxide (0.5 mM), glucose (55 mM) or calcium (15 mM) in incubation media. Hydrogen peroxide induces cataract by oxidative damage of lens protein. A high concentration of glucose leads to the accumulation of sorbitol in lens producing osmotic stress and high concentration of calcium ion causes the precipitation of lens protein and subsequent lens opacity (cataract formation). The time required for cataract development is usually 24–48 h in this model.

Method: Eye lenses are isolated from rats, goats, or pigs and transferred into incubation media like Tyrode solution. The fresh lenses are selected and divided into different groups: normal, control, and test group. The normal lenses are placed in incubation media only. The control lens is placed in incubation media containing inducing agent, hydrogen peroxide (0.5 mM), glucose (55 mM) or calcium chloride (10 mM), while test lenses are placed in incubation media containing inducing agent with test drug. The time required to complete opacity and lens biochemical parameter is observed. The test results are compared with control group.

Interpretation: If the test drug increases the time required for complete opacification it is considered as an anticataract agent. The anticataract also restores the lens protein, ions, and antioxidants level as compared to control group (Bodake et al., 2012; Chandorkar et al., 1981; Umamaheshewari et al., 2012).

Model Used for Retinopathy:

Diabetic retinopathy is one of the most common microvascular complications of diabetes mellitus. Many factors are involved in the pathogenesis of DR such as metabolic disorders like hyperglycemia, high blood pressure, hyperlipidemia, age and oxidative stress. Pathologically, human diabetic retinopathy is classified into progressive stages, namely nonproliferative, pre-proliferative and proliferative retinopathy. Neovascularization (new blood vessel formation) accompanied by bleeding and tractional retinal detachment is the characteristic feature of retinopathy that is observed by ophthalmoscopy (Choudhary et al., 2017).

DIABETIC-INDUCED RETINOPATHY

Diabetic rats develop most of the early stage lesions of retinopathy, however, advanced lesions are not faithfully produced. This animal model is particularly useful in the study of early retinal functional and

structural changes and biochemical abnormalities. Microangiopathic lesions have been observed in rats with streptozotocin (STZ) induced or with spontaneous diabetes. STZ and galactose induced diabetic retinopathy is the most common animal model used to assess the drugs that reduce the progression of retinopathy.

Method: Albino mice (25–30 g) and rats (150–200 g) are commonly used in this model. Diabetic retinopathy in animals is induced by single intraperitoneal injection of STZ (65 mg/kg). After one week blood glucose level is measured in each animal and the animals with high glucose concentration (>165 mg/dl) are considered as diabetic rats. Thereafter animals are divided into diabetic control (n=5–6) and test treatment group (n=5–6). The diabetic control group is treated with saline and test group is treated with test drug for 12–15 weeks. The normal group (n=5–6) is also taken and treated with saline only for 12–15 weeks. Morphology of eyes is monitored weekly by using ophthalmoscope. And after completion of the protocol animals are sacrificed and the retinal microvascular lesion is histopathologically examined.

Interpretation: STZ treatments in diabetic control group increase the progression of retinopathy lesion as compared to the normal group while treatments with test drug that reduces the progression of retinopathy lesion as compared to diabetic control (Gong et al. 2013; Lai and Lo, 2013), can be considered for the treatment of retinopathy.

LOCAL ANAESTHETICS

Local anesthetics are the drugs that cause the reversible loss of sensory perception by inhibiting block voltage-sensitive sodium channels and inhibit the generation of the action potential. Thus, local anesthetics block the nerve conduction by decreasing the entry of Na^+ during action potential generation. Clinically it is used to relief pain and in minor surgical procedure. There are several local anesthetics available such as cocaine, procaine, lidocaine, tetracaine, etc. Local anesthetics are either applied topically on the desired surface area (surface anesthesia) or injected subcutaneously (infiltration anesthetic effects (Brunton et al., 2006).

MODELS FOR SCREENING LOCAL ANESTHETICS

Several experimental animals like rats, guinea pigs, rabbit, and frogs are employed to screen local anesthetics. The models (Table 25.1) are developed for screening of local anesthetic based on its effects, surface anesthesia, infiltration anesthesia, and conduction anesthesia

Sr. No.	Screening Models	Description
I	**In Vivo Models**	
A	Conduction anesthesia in rat's sciatic nerve	Albino rats are used in this model. The sciatic nerve located in the leg is blocked by administration of a local anesthetic solution. The effect is observed as depression of walking behavior due to nerve conduction block.
B	Conduction anesthesia on mouse tail	The tail flick or radiant heat method is used in this test. Administration of local anesthetic at the root of the mouse tail increases the reaction time to thermal stimulus.
C	Retrobulbar block in dogs	Young female mongrel dogs (13–15 kg) are used. Retrobulbar injection of local anesthetic is given behind the eye and the anesthetic effect is observed through ocular responses such as miosis.
D	Infiltration anesthesia in guinea pigs	The front and back portions of the guinea pig's skin are shaved and wheals are prepared. Local anesthetic is applied to the wheal area, and response to pin prick is tested on both wheal and normal areas. The drug decreases the pin-prick response in the wheal area.
E	Surface anesthesia on cornea of rabbit	Local anesthetic is applied to the rabbit's eye and corneal reflexes are observed. Local anesthetics block or reduce the corneal reflex response.
F	Spinal anesthesia in rats	Albino rats are used to study spinal anesthesia. Local anesthetic solution is injected intrathecally at the lumbar (L5–L6) region using a 30-gauge needle at about a 20° angle. The anesthetic effect is evaluated by observing tail flick response to radiant heat.

<u>In Vitro models</u>

Isolated sciatic nerve preparation

The isolated sciatic nerves from guinea pig or frog are used in this model. The administration of local anesthetics retards the action potential generation.

Voltage clamp experiments

Voltage clamp experiments are used to determine sodium and potassium conductance. Single myelinated nerve fibers from the sciatic nerve of the frog are used in this study. Local anesthetics decrease the sodium

and potassium ion current.

IN VIVO MODEL

Conduction anesthesia in rat's sciatic nerve

Based on the previous studies, Camponigri and Tahran (1971) describe the method of conduction anesthesia in rat's sciatic nerve. The local anesthetics block the nerve conduction and modulate the ionic channel duration of cellular function. Vasoconstrictor (e.g. noradrenaline) can be mixed with a local anesthetic to enhance anesthetic effects. Albino rats, guinea pigs and frog model used often create anesthesia in the sciatic nerve. Guinea pig represents peripheral and intrathecal anesthesia in man.

Method: Male albino rats (125–175 g) are used in this model. Experimentally, animals are poised in a prone position (ventral side is down), hind limbs are extended, and the depression for needle insertion is located by palpation with the left index finger. 0.2 ml of 1% (w/v in saline) test local anesthetic or standard drug (e.g., procaine or lidocaine) is injected to block the sciatic nerve in the junction of the biceps femoris and the gluteus maximus muscles of legs. Tuberculin syringe with 24–25-gauge needle is used to administration of the drug. One leg is used for a test drugs administration and other leg is used for vehicle administration. Immediately after the injection, repeated checks of the digit of the foot and the walking behavior are monitored in every 5 to 10 min time interval to determine the recovery process.

Interpretation: Average onset and duration of action are calculated. The frequency of blocks is noted. The different doses of test compound and standard are used to establish potency ratio by dose response curves. The digits of the foot are wide apart in the normal rats (vehicle treated only). Whereas, in the test or standard drug treatment (the blocked leg), digits of the foot are closed together. The walking behavior performances are also depressed as indicated by dragging of the leg and an inability to walking. After the time of recovery the block for each leg is noted, each animal is examined every 5 to 10 min in order to note the time of recovery (Camougis and Takman, 1971).

Modified methods of sciatic nerve block technique

The previous sciatic nerve block technique is modified by applying additional stimulation to generate action potential in the albino rats. A 16 mm long 25-gauge hypodermic needle is inserted percutaneously into the sciatic notch situated between greater trochanter and the ischial tuberosity, pointing towards the ischium. A lead clip is attached to the top of the needle and stimulating pulses (0.3 v, 2 ms, 2 Hz, negative polarity) are delivered. After ipsilateral hindleg kick was observed, 0.5 ml of 1% vehicle/test/standard

drug solution is injected over 5 s and the needle was removed after an additional 5 s. The duration of blockading and recovery time is observed. The local anesthetics increase the duration of block time as compared to vehicle treated animals (Kroin et al., 2004; Thalhammer et al., 1995).

B. **Conduction anesthesia on the mouse tail:**

The mouse tail flick method is generally used for evaluation of the systemic analgesic activity. This method can also be used for determination of anesthesia property (conduction anesthesia) by injecting the local anesthetic into the root of the tail. This model is used to quantify the duration of conduction block by local anesthetics (Grant et al 1993).

Method: Albino mice 22–25 g are used in this method. Before injecting vehicle/ test/standard drug the normal reaction time is noted against radiant heat (pretreat value). The animals which show reaction time of more than 6 sec are not used for the test. The maximum time mouse can be kept under the radiant heat is observed (cut off time). Experimentally animals are divided into three groups: control (vehicle), test, test standard drugs, and standard (standard drug eg. procaine, tetracaine). Animals are placed in restrainer (animal holder with tail opening) and vehicle/test/standard drug (0.1 ml) is injected on both sides of the area of the tail root in the respected group. After 10 min of injection, animals are subjected to radiant exposure (radiant heat is exposed to the proximal third of the tail. The area of heating is about 1.5 cm distal to the injection site. For each individual animal, the reaction time is noted before (pretest) and after treatments.

Interpretation: The average reaction time is calculated and compared with pretest value. Percentages of positive animals (those showed higher reaction time than pretest) are counted for each dose and ED_{50} values are calculated according to LITCHFIELD and WILCOXON methods (Grant et al. 1993).

Apart from the radiant heat technique, tail-pinch method is also applied to determine the local anesthetic property. The vehicle or local anesthetic drug is injected subcutaneously bilaterally at the root of the tail. After 15 min of injection, tail is pinched by a polyethylene sheathed artery forceps and repeated in every 15 min. The number of animals failing to remove the forceps by biting is recorded and compared with vehicle treated animals (Madan et al. 1970).

C. **Retrobulbar block in dogs**:

Defalque and Stoelting (1976) describe the method to determine latency and duration of action of local anesthetics after retrobulbar injection in dogs. After retrobulbar injection of local anesthetics, miosis

effects are observed. In the retrobulbar space, local anesthetics provide akinesia of extraocular muscles by blocking cranial nerves (ophthalmic and maxillary branches), thus globe movements are blocked. Retrobulbar block also provides sensory anesthesia of the cornea, uvea, and conjunctiva by blocking the ciliary nerves.

Method: Young female mongrel dogs (13–15 kg) are used in this model. One day before the test, eserine ointment (0.25%) is applied in each conjunctival sac of the dog. Animals are anesthetized by administration of pentobarbital (25 mg/kg i.v.), then light anesthesia is maintained by repeated with 10 mg/kg at hourly intervals. Ten min after i/corneal reflex present, the dog is kept into 30-degree head-down position, and 20 ml of 0.05% tetracaine is forced into the epidural space through the interarcuate ligament. After 5 min Horner's syndrome is observed – it is characterized by miosis (a constricted pupil), partial ptosis (a weak, droopy eyelid), apparent anhidrosis (decreased sweating), with or without enophthalmos (inset eyeball).

A surgical lamp with the 150 watt bulb is focused on the eye from a one meter distance. Fifteen min later, a retrobulbar block is performed. An ophthalmic forcep is used to seize the sclera and the eyeball is pulled downward and medially. A needle (23-gauge) is then inserted through the superior rectus muscle tangentially to the globe, and is immobilized as soon as a click indicates penetration of the retrobulbar space. 2 ml of vehicle (control) or test drug is then administered (0.5 ml/sec after aspiration. Pupil dilates and reaches its maximal diameter (5 mm) within a few minutes after injection of test local anesthetic. This apparent diameter is estimated with a 2 cm long ruler calibrated in millimeters, whose center is gently applied to the corneal center. The pupil is observed every 15 sec for 5 min, then every 5 min until the reappearance of maximal miosis (pinpoint and asymmetrical), a precise endpoint, which generally coincides with corneal reflex and lacrimation.

Interpretation: The drug latency (in min) and duration (in 5-min units) are averaged for both eyes of each animal, and the mean values are compared with control (vehicle treated) one (Defalque and Stoelting, 1967; Vogel, 2002).

D. **Infiltration anesthesia in guinea pig's wheals:**

This method has become a standard operating procedure for testing local anesthetics. Based on studies, the use of intracutaneous wheals in guinea pigs is recommended for this study.

Method: Adult guinea pigs of either sex (250–300 g) are used in this model. Animals are acclimatized one day before the test by shaving 4–5 cm area on back and front portion. Front area of skin is more sensitive

than back area. Therefore, each concentration of a local anesthetic must be tested in both areas simultaneously. Three animals are used for in each group, one dose is given in front and another dose is given in back area. The test or standard drug is injected intracutaneously in 0.1 ml saline. After injection, size of the wheal is marked with ink. One side is used for the test drug preparation, the other side for the standard (e.g., 1% butanilcaine). The reaction to pin prick is tested 5 min after injection. The normal reaction is observed by pricking outside the wheal. In the wheal region, six pricks are applied at 3–5 sec. Six pricks are applied every 5 min for 30 min. After completion, the test is repeated in 3 other guinea pigs, but the solution, which was used for the front, is now used for the back area and vice versa.

Interpretation: Calculate the sum of a number of pricks fail to elicit a response during the 30 min period (36 pricks). Using various doses, dose response curves can be established. The mean values of the test are compared with standard and normal. The local anesthetic drugs increase the number of prick fails to elicit a response (Bülbring and Wajda, 1945).

E. **Surface anesthesia on the cornea of rabbits:**

Rabbits are used to assess surface anesthetic effects of a local anesthetic by local administration in conjunctival sac of one eye and corneal reflexes are observed.

Method: Albino rabbits (2.5–3 kg) are placed into rabbit holding cages. The upper and lower eyelashes are carefully clipped. 0.5 ml of vehicle/test anesthetic/standard drug is applied into the conjunctival sac of one eye for 30 s by using 22 gauge needle in their respective group. Thereafter, another dose (0.1 ml) is added within 1 min. 0.1% solution of tetracaine hydrochloride is used as a standard drug. Corneal reflex (blinking) is triggered by any touch of the cornea. For quantitative estimation, the irritation with a needle according to has also been recommended. In a glass rod, an unique hair bending at a load of 230 mg is attached perpendicularly and used to stimulation. Within 25 s, the cornea is touched 100 times. The test is started after 5 min application of the test/standard drug and repeated every 5 min until anesthesia disappeared and reflexes (blinking) occurs again. The time between disappearance and reappearance of the corneal reflex is monitored.

Interpretation: The local anesthetics increase the time of reappearance of the corneal reflex. At different doses, dose-response curves can be established and potency ratios versus the standard is calculated (Gerlough TD, 1931; Vogel, 2002).

Apart from the above methods, spinal anesthesia in rat test can also be used to screen local anesthetics.

25.4 **IN VITRO MODEL**

A. **Isolated sciatic nerve preparation of the frog:**

Isolated sciatic nerve preparation from frog, guinea pig, and mammalian is used to assess local anesthetic effects. Isolated sciatic nerves are placed between stimulating and recording electrodes in solutions containing a local anesthetic, and observed electrophysiological changes (Paterson and Hamilton 1970).

Method: Frog is sacrificed by decapitation. The abdomen and hind legs are skinned by peeling the skin in a posterior direction. The sciatic nerves are exposed by cutting thigh muscles. Finally, the gastrocnemius muscle is removed and the peroneus muscle is extended laterally from the tibiofibular. The entire sciatic nerve with branches should now be exposed in the sacral vertebral region, the nerve is tied with the help of thread. The nerve is then cut anterior to the thread. The nerve is kept moist with frog Ringer solution during the isolation procedure. The connective tissue and the nerve branches are cut carefully. Near the distal end of the tibiofibular the nerve is cut and extended on a filter paper previously soaked with Ringer solution. The distal end of the nerve is tied with a length of thread. The sciatic-peroneal nerve preparation is mounted in a nerve chamber for recording. Stimulator electrode is placed on the proximal end of the nerve and at the distal end recording electrodes are placed. The isolated nerve is immersed in frog Ringer solution. In the nerve stimuli are applied (5–15 mV, frequency 30% and duration 5 ms) by the commercial stimulator e.g. Grass Model S4. Stimulation of the nerve produces a display on the oscilloscope; voltage is adjusted on the stimulator will cause the action potential to increase and decrease correspondingly in the scope. The action potential should be monophasic and diphasic injury above the baseline after touching the nerve between the last two recording sites, the spike is recorded frequently for several minutes so that the spike is stable. The physiological salt solution is added to a trough containing the local anesthetic dissolved in Ringer solution. At first, plain Ringer, the amplitude of the spike is recorded and recorded on a polygraph or on a scope. Then drug solution, the drug solution is removed and the nerve is washed in this Ringer solution. Amplitude are the values recorded.

Interpretation: With the different dose concentration of test and standard, block and recovery curves are achieved and dose-response curves can be calculated. The local anesthetic drugs slow down the recovery process (Paterson and Hamilton 1970; Vogel, 2002).

B. **Voltage clamp experiments for sodium and potassium conductance:**

Single myelinated nerve fibers from the sciatic nerve of the frog are used in this study. Voltage clamp experiments on single nodes of Ranvier can be performed with single myelinated nerve. Sodium,

potassium and leakage currents (I_{na}, I_k, I_l) can be measured. Local anesthetics generally block the voltage-gated cation channels.

Method: Single myelinated nerve fibers are dissected from the sciatic nerve of the frog (Rana esculenta). The fibers are immersed in a Ringer solution (110 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 5.0 mM Tris, pH 7.3, at 15 °C). The nodal membrane is clamped to a holding potential of –30 mV (relative to the resting potential of –70 mV) for measurement of sodium, potassium and leakage currents (I_{na}, I_k, I_l). Hyperpolarizing pre-pulses (–40 mV, 50 ms) and afterward test pulses (2 ms, 60 mV) are applied in every 5 s to record peak sodium currents (I_{na}) and to avoid the influence of accumulation of frequency-dependent block. Every second pulse is followed by an afterpulse (120 mV, 20 ms) to measure steady state I_k. The leakage current, I_l, is measured at the end of the hyperpolarizing prepulse and recorded every 10 s.

Interpretation: After superfusion of the local anesthetic with the nodal membrane, a quick and then a slow decrease of the peak sodium current (I_{na}) is observed. When the local anesthetic application is withdrawn, sodium current (I_{na}) increases quickly and then slowly within minutes indicating a reversible action of the local anesthetic on the nerve fiber. On the application of local anesthetics, potassium current (I_k) behaves similarly, while the leakage current (I_l) remains unaffected.

IMPORTANT QUESTIONS

Very Short Questions (2 marks each)

1. What is a sympathomimetic drug?
2. Define parasympatholytic.
3. Name one skeletal muscle relaxant.
4. What is the primary action of local anesthetics?
5. Mention one drug that acts on the eye.
6. What does a sympatholytic drug do?
7. Define parasympathomimetic.
8. Give an example of a local anesthetic.
9. What is the primary use of drugs acting on the eye in preclinical studies?
10. Name a drug that acts as a skeletal muscle relaxant.

Short Questions (5 marks each)

1. Describe the mechanism of action of sympathomimetic drugs.
2. Explain the effects of parasympatholytics on the autonomic nervous system (ANS).
3. Discuss the role of skeletal muscle relaxants in preclinical studies.
4. Summarize the actions of drugs acting on the eye and their importance in preclinical research.
5. Illustrate the method of evaluating local anesthetics in animal models.
6. Compare sympathomimetic and sympatholytic drugs in terms of their actions and applications.
7. Explain the use of parasympathomimetic drugs in preclinical screening models.
8. Describe the screening process for skeletal muscle relaxants in animal models.
9. Discuss the importance of local anesthetics in preclinical studies.
10. Explain the significance of using animal models to study drugs acting on the eye.

Long Questions (10 marks each)

1. Analyze the screening models for sympathomimetic and sympatholytic drugs, highlighting their mechanisms and evaluation criteria.
2. Evaluate the preclinical screening models for parasympathomimetics and parasympatholytics, discussing their significance and challenges.
3. Critically assess the various screening methods for skeletal muscle relaxants, including methodology and expected outcomes.
4. Discuss the comprehensive screening models for drugs acting on the eye, focusing on the procedures and expected results.
5. Describe the preclinical screening process for local anesthetics, including preparation, methodology, and evaluation.
6. Compare and contrast sympathomimetics and parasympathomimetics in terms of their actions, applications, and screening models.

7. Evaluate the role of animal models in studying the effects of skeletal muscle relaxants and their implications for human use.
8. Discuss the advancements in screening models for local anesthetics and their impact on preclinical research.
9. Analyze the challenges and ethical considerations in the preclinical screening of drugs acting on the ANS.
10. Discuss the significance of preclinical screening models for ANS activity in the development of new therapeutic agents.