Chapter-III
Material and Instruments
Preparation of reagents and solutions
Section (i): Preparation of Reagents and Solutions:

AR Grade Chemicals are used for preparation of Reagents and solutions in the present investigation.

**Buffer solution (p^H 1.5):**

Buffer solution is prepared by mixing 289 ml of glycine solution (37.52 gm of glycine and 29.24 gm of NaCl are dissolved in 500ml of distilled water) with 711ml of 0.1 M Hcl.

**Wool fast blue solution: (0.2% W/V):**

Wool fast blue solution is prepared by dissolving 200mg of wool fast blue (Flaka) in 100 ml of distilled water.

**0.1% 2,3 dichloro-dicyano 1,4 Benzoquinone solution(DDQ):**

100 mg of DDQ is dissolved in 100 ml chloroform to obtain the 0.1% DDQ solution.

**Escitalopram solution:**

50 mg of pure escitalopram solution is dissolved in methanol and the volume is adjusted to 50 ml with methanol. The stock solution is further diluted to get working concentration of 100 μg/ml.
**Nortriptyline solution:**

50 mg of nortriptyline solution is dissolved in 50 ml methanol. 1.0 ml of the above stock solution is further diluted to 50 ml with methanol to get working concentration of 100 μg/ml.

**Primaquine solution:**

Pure primaquine solution (50 mg) is dissolved in 50 ml methanol. Further 1 ml of the stock solution is further diluted to 50 ml with methanol to get working concentration of 100 μg/ml.
A Brief profile of selected drugs
Section (ii): Brief profile of selected drugs

(a) Escitalopram:

Escitalopram is chemically, (1S)-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-3H-2-benzofuran-5-carbonitrile. Chemical formula is C\textsubscript{20}H\textsubscript{21}FN\textsubscript{2}O. Escitalopram is one of a class of antidepressants known as selective serotonin reuptake inhibitors (SSRIs). It is used to treat the depression associated with mood disorders. It is also used on occasion in the treatment of body dysmorphic disorder and anxiety. The antidepressant, antiobsessive-compulsive, and antibulimic actions of escitalopram are presumed to be linked to its inhibition of CNS neuronal uptake of serotonin. \textit{In vitro} studies show that escitalopram is a potent and selective inhibitor of neuronal serotonin reuptake and has only very weak effects on norepinephrine and dopamine neuronal reuptake. Escitalopram has no significant affinity for adrenergic (alpha\textsubscript{1}, alpha\textsubscript{2}, beta), cholinergic, GABA, dopaminergic, histaminergic, serotonergic (5HT\textsubscript{1A}, 5HT\textsubscript{1B}, 5HT\textsubscript{2}), or benzodiazepine receptors; antagonism of such receptors has been hypothesized to be associated with various anticholinergic, sedative, and cardiovascular effects for other psychotropic drugs. The chronic administration of escitalopram was found to downregulate brain norepinephrine receptors, as has been observed with other drugs effective in
the treatment of major depressive disorder. Escitalopram does not inhibit monoamine oxidase. Mechanism of Action The antidepressant, antiobsessive-compulsive, and antibulimic actions of escitalopram are presumed to be linked to its inhibition of CNS neuronal uptake of serotonin. Escitalopram blocks the reuptake of serotonin at the serotonin reuptake pump of the neuronal membrane, enhancing the actions of serotonin on $5\text{HT}_{1A}$ autoreceptors. SSRIs bind with significantly less affinity to histamine, acetylcholine, and norepinephrine receptors than tricyclic antidepressant drugs. Chemical structure of escitalopram is shown in fig:3.2.1.

![Chemical structure of escitalopram](image)

**Fig:3.2.1: Escitalopram**

Uses: It is used to treat the depression associated with mood disorders. It is also used on occasion in the treatment of body dysmorphic disorder and anxiety.
Doses: Adults 20 mg daily. Articalm tab 10 mg, Esdep Tab 20 mg, Excita Tab 10 mg, Jolivel Tab 5 mg etc are available in the markets. Absorption The absolute bioavailability of escitalopram is about 80% relative to an intravenous dose.

Adverse effects: Toxicity Signs of overdose include convulsions, coma, dizziness, hypotension, insomnia, nausea, vomiting, sinus tachycardia, somnolence, and ECG changes (including QT prolongation). Protein Binding ~56% Biotransformation Mainly hepatic

(b). Nortriptyline

Nortriptyline chemically, \(3-(10,11\text{-dihydro-5H-dibenzo}[a,d]\text{cyclohepten-5-ylidene})\)-N-methyl-1-propanamine. The chemical formula of nortriptyline is \(C_{19}H_{21}N\). Similar to protriptyline, nortriptyline is a tricyclic antidepressant of the dibenzocycloheptene type and is the active metabolite of amitriptyline. It is believed that nortriptyline either inhibits the reuptake of the neurotransmitter serotonin at the neuronal membrane or acts at beta-adrenergic receptors. Tricyclic antidepressants do not inhibit monoamine oxidase nor do they affect dopamine reuptake. Chemical structure of nortriptyline is shown in fig:3.2.2.
Fig:3.2.2: nortriptyline

Uses: It is used as an antidepressive agent. It is also used in major depression, dysthymia, and atypical depressions.

Doses: 30-50 mg daily in divided doses. Max doses 150 mg. Nortriptyline are available in different trade names in the market such as noritren, nortrilen, nortriptyline Hcl, nortryptiline, norzepine, Nordep, nortin, primox etc.

Adverse effects: Symptoms of overdose include cardiac dysrhythmias, severe hypotension, shock, congestive heart failure, pulmonary edema, convulsions, and CNS depression, including coma. Changes in the electrocardiogram, particularly in QRS axis or width, are clinically significant indicators of tricyclic antidepressant toxicity.
Primaquine chemically, N-(6-methoxyquinolin-8-yl)pentane-1,4-diamine. The Chemical formula of primaquine is $\text{C}_{15}\text{H}_{21}\text{N}_{3}\text{O}$. Primaquine is an antimalarial agent and is the essential co-drug with chloroquine in treating all cases of malaria. In the blood, malaria parasites break down a part of the red blood cells known as haemoglobin. When this happens haemoglobin is divided into two parts; haem and globin. Haem is toxic to the malaria parasite. To prevent it from being damaged, the malaria parasite produces an chemical which converts the toxic haem into a non-toxic product. Primaquine acts by interfering with a part of the parasite (mitochondria) that is responsible for supplying it with energy. Without energy the parasite dies. This stops the infection from continuing and allows the person to recover. Primaquine kills the intrahepatic form of *Plasmodium vivax* and *Plasmodium ovale*, and thereby prevents the development of the erythrocytic forms that are responsible for relapses (it also kills gametocytes). Primaquine is not used in the prevention of malaria, only in the treatment. It has insignificant activity against the asexual blood forms of the parasite and therefore it is always used in conjunction with a blood schizonticide and never as a single agent. Primaquine has gametocytocidal
activity against all plasmodia, including *P. falciparum*. The Chemical structure of primaquine is shown in fig:3.2.3.

![Chemical structure of primaquine](image)

**Fig:3.2.3: primaquine**

**Uses:** Primaquine is an antimalarial agent and is the essential co-drug with chloroquine in treating all cases of malaria.

**Doses:** Adults 30-p45 mg/day during the last two weeks of chloroquine prophylaxis. Leoprime Tab 2.5 mg (Kid), Tab 7.5 mg, Malirid Tab 7.5 mg Pqine Tab 15 mg are available in the markets.

**Adverse effects:** Adverse effects include anemias and GI disturbances.
Brief description of instruments employed.
Section(iii): Brief description of instrument employed:

The brief description of Milton Roy Spectronic 100 plus, Spectrophotometer is employed in the present investigation is given in this chapter.

Spectrophotometer:

Spectronic 1001 plus Spectrophotometer model No.335002 with battery back up test memory is used for all the Spectrophotometric studies. The instrument provides a unique monochromatic design and a variety of micro process controlled features to give fast and accurate spectrophotometric measurements.

The instrument contains a superior optical system, which splits of a small fraction of light beam and uses it as a reference beam to achieve a high level of stability. High intensity deuterium and tungsten-halogen lamps, silica coated steroidal mirrors and Milton Roy own blazed holographic grating produced exceptional energy and spectral purity. It has a 2 nm spectral bandwidth, which provides high re-solution sample measurements. This means that we can have precise and accurate results for an extensive range of samples and test procedures time after time. Further the instrument can be used for increased reproducibility by automating the testing with
programmed test formats because the test parameters can be stored and recalled quickly and easily.

Spectronic 1001 plus is easy to use because the soft touch keyboard is remarkably responsive and well organized. A full alpha numeric display gives us messages step by step to guide and help us during set up and testing. This means that the instrument alerts us to any incorrect entries. The microprocessor control enhances reliability of the results. Once the tests are programmed they can be run on sample after sample with no deviation. The spectronic 1001.Plus is preprogrammed for a variety of test mode operations and applications.

The Spectrophotometer is useful to perform a variety of test models and functions. The instrument displays the name of each test mode or function with its assigned number. It has three distinct segments of memory namely non-erasable memory, long-term memory and current memory. The Spectrophotometer recognizes two types of numerical entries namely the prompted entries and the unprompted entries. Entries made while editing the parameters of a test are called prompted entries and all other numerical are called unprompted entries. Thus the instrument has a number of special programming options, functions, test modes and parameters setups etc.
For optical operation of the instrument, it is allowed to warm on 30 minutes prior to operation and then another 15 minutes are allowed as warm up period whenever a lamp is first turned on. Once the proportional sequence and the instrument are warmed up the spectrophotometer is used to analyze the samples. The instrument can express the results of analysis in any of three data modes namely absorbance, transmittance or concentrations. Thus the instrument Spectronic 1001 plus gives us precise and accurate results and is one of the well-recognized instruments in the laboratories throughout the world for its superior and data handling capabilities.