2. Literature review

2.1. Gaozaban

Botanical Name: *Onosma bracteatum* Wall  
Family Name: Boraginaceae  
English name: Sedge  
Unani Name: Gaozaban  
Local Name: Telsharong, Gojihva, Ratanjot  

Gaozaban in unani system of medicine and Sedge in Middle East. According to the early literature, the dried leaves and flowers of this plant constitute the drug Gaozaban, which was imported from Iran. It is found abundantly in Northwestern Himalayas from Uttar Pradesh to Kashmir up to 3,500 - 4,500 meters in height. It is perennial growing to 0.4m.

![Onosma bracteatum Plant](image1)

Fig:2.1 *Onosma bracteatum* Plant

![Gaozaban leaves](image2)

Fig:2.2 Gaozaban leaves
Chapter 2

Literature review

The stem is simple, hairy, arising from a cluster of radical leaves, which are lanceolate and with conspicuous hairy pallid bases. The leaves are with evident veins. The cauline leaves are lanceolate. The flowers are hermaphrodite (have both male and female organs) yellow in colour, trumpet-shaped, in dense, silky, glomaerate clusters and are pollinated by Insects. The nut-lets are grey, coarsely rugose and tuberculate.

The drug is used as diuretic and is considered cooling. It is useful as a spasmolytic. Sedge has been traditionally used as a tonic that helps build the body's immune resistance and regulate urine output. The plant is alterative, demulcent, refrigerant and tonic. A decoction is used in the treatment of rheumatism, syphilis and leprosy. The plant is considered to be useful in relieving excessive thirst and restlessness in febrile excitement, and also to be useful in relieving functional palpitation of the heart, irritation of the bladder and stomach, and strangury (Chopra et al, 1986). It has been advocated in use of variety of ailments, including asthma by stabilizing mast cell activity (Choudhary, 2010), and also reported as antiallergic and anti-inflammatory activity (Patel et al, 2011). One of the studies demonstrated its significant role in the marked reduction of bronchial hyper-responsiveness by decreasing infiltration of oesinophils and neutrophils in rodents (Patel et al, 2008).
2.2. Miswak

Botanical Name: *Salvadora persica* Linn
Family Name: Salvadoraceae
Local Name: Miswak, Meswak, pilu, Chewing stick

The medicinally important species of “*Salvadora persica*” Linn belong to family of Salvadoraceae. The tree drives its Persian name, Darakht-e-miswak or tooth brush tree, from the fact that wood is much employed for the manufacturers of tooth brush. This plant have geographical range extending from Central Africa, Egypt, Saudi Arabia, Yemen, South-western Asia to India, Pakistan, Sudan and Tanzania. It is a large much-branched, evergreen shrub or a tree. Bark is dull grey or grey-white deeply cracked and leaves are variable in shape—elliptic-ovate-lanceolate—somewhat flashy. Flowers are pedicellate, greenish-white or greenish-yellow in lax panicles, drups are globose or round, smooth, red when ripe. The trees readily regenerate from seeds and coppice well (Anonymous CSIR, 1972).

![Fig:2.3 Branches of *Salvadora persica*](image1)
![Fig:2.4 Miswak](image2)

A phytochemical investigation of stems from *S. persica* by Khalil (2006) resulted in the first isolation of four benzylamides from a natural source.
The isolated compounds were identified as butanediamide, $N^1, N^4$-bis (phenylmethyl)-2(S)-hydroxy- butanediamide (I), $N^-$-benzyl-2-phenylacetamide (II), N-benzylbenzamide (III), and benzylurea (IV) (Khalil, 2006). Phytochemical investigation revealed that it contains oleic, linolic, and stearic acids. Among the compounds identified are esters of fatty acids and of aromatic acids, and some terpenoids (Howaida et al, 2003). The major components from the essential oil of the toothbrush tree miswak stem have been identified as 1,8-cineole (eucalyptol) (46%), $\alpha$-caryophellene (13.4%), $\beta$-pinene (6.3%), and 9-epi-(E)-caryophellene (Alali and Al-Lafi, 2005). GC-MS analysis of the volatile oil extracted from miswak tree leaves revealed benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene, and $\alpha$-caryophyllene as important constituents (Alali et al, 2003). Sticks from miswak have been analyzed for their soluble and total content of fluoride, calcium, phosphorus, and silica. There was a substantial amount of silica in the ashes of miswak (Hattab, 1997). The aqueous extract of stem and root of miswak tree has also been investigated for some antimicrobial anionic components by using capillary electrophoresis techniques. It was reported that the root and stem extracts contain sulfate chloride, thiocynate, and nitrate (Darout et al, 2000). Physicochemical analysis of air-dried root bark of miswak tree was carried out by Bhandari in 1990. It contains 27.1% ash, consisting of considerable amounts of salts, mostly as chlorides. The drug has large amount of alkaloidal constituents (including trimethyl amine and unidentified alkaloids), small amount of resin and coloring matter, and traces of tannins and saponins. Higher concentration of fluoride and silica, sulfur, vitamin C, small amount of flavonoids and sterols were also reported (Bhandari, 1990, Kirtikar and Basu, 1987). Three lignin glycosides have been reported from the stem of S. persica (Kamal et al, 1992). The flavonoids rutin and quercetin were
detected in the stem of *S. persica* (Abdel-Waheb et al, 1990). Salvadourea has been reported in the root of *S. persica* (Ray et al, 1975). Benzylisothiocynate was also isolated from the root (Al-Bagieh, 1990). Salvadoricine, a new indole alkaloid, was reported in the leaves of *S. persica* (Malik, 1987).

All part of the plant like root, shoot, leaves, bark and fruits are used as a medicinal purpose. For over a thousand year, miswak has been commonly used as a chewing stick by the peoples of Africa, Saudi Arabia, Yemen, India, Pakistan etc. It has various properties like anti-bacterial, anti-inflammatory, anti-rheumatic, aphrodisiac, expectorant, diuretic, anthelmintic and astringent. It is also used in the treatment of asthma and bronchitis and used to strengthen the teeth, tonic to the liver and improve the digestion and improve the appetite (Chopra et al, 1956, Anonymous, CSIR, 1972, Krithikar and Basu, 1975, Vaidyarathnam, 1990).

Pure miswak extract has a wide spectrum of benefit from head to toe (including teeth and gums). The peoples of Arabia believe some of these popular beliefs which are:

Ensures safe passage on the holy bridge of “Siraat”, Angels are happy with all those who use “Miswak”, The rewards of good deeds increases many fold, Scares away “Sataan”, User gets the benefit of remembering the “kalmia” in his last days, clean the mouth and teeth, strengthen the gums, reduces the tooth ache, act as a breath freshener, increase the brightness of eyes, enhances vision, improves memory and prevents early aging.

Salehi Sourmaghi et al. (1996) reported that the chewing stick or Miswak has been used by many cultures from the ancient times as tooth brush to promote oral health and hygiene.
Pharmacological Profile

Antimicrobial activities
Aqueous and methanol extracts of *S. persica* were investigated by Firas et al. (2008) for its antimicrobial activities against seven isolated oral pathogens like Staphylococcus aureus, Streptococcus mutans, Strep. faecalis, Streptococcus pyogenis, Lactobacillus acidophilus, Pseudomonas aeruginosa, and Candida albicans using disc diffusion and microwell dilution assays. The strongest antibacterial activity was observed using the aqueous extract against Streptococcus. Miswak (*S. persica*) extract inhibits the growth of some dental plaque bacteria, and antibacterial effect of the herbal toothpaste was significantly greater than that of the placebo (Poureslami et al., 2007). Aqueous extracts of miswak enhance the growth of fibroblasts and inhibit the growth of cariogenic bacteria (Darmani et al., 2006).

Antidental caries potential
The efficacy of natural tooth brush or miswak in the prevention of dental caries has been investigated and compared with the efficacy of ordinary toothbrush and toothpaste. Rinsing with miswak extract (*S. persica*) stimulated parotid gland secretion and raised the plaque pH, suggesting a potential role in caries prevention (Sofrata et al., 2007).

Antiplaque activity
It has been observed that miswak was as effective as a toothbrush for reducing plaque on buccal teeth surfaces both experimentally and clinically (Mohammed et al., 2006).

Antimycotic potential
Al-Bagieh et al. (1994) showed that miswak extract at a concentration of 15% and above has a fungistatic effect for up to 48 hours. The antimycotic effect was probably due to one or more of the root contents which included chlorine, trimethylamine, and alkaloid resin, and sulfur.
compounds.

**Anti-inflammatory and analgesic potential**

Mansour et al. (1996) evaluated the extract of root and branches of *S. persica* for analgesic activity in mice and found that the drug possesses a relatively moderate analgesic effect which might be due to interaction with the central and/or peripheral opiate system. The extract of stem of *S. persica* has been reported to possess anti-inflammatory activity (Ezmiril et al, 1979, Rajesh et al, 2010).

**Antiulcer activity**

The antiulcer activity of decoction of miswak has been reported against aspirin induced ulcer in rats. The ulcer index significantly decreased after the treatment with a lyophilized decoction of miswak (500 mg/kg, os), once daily for 7 days, in comparison to controls. Moreover, *S. persica* decoction possesses significant anti-inflammatory activity (Sanogo et al, 1999). The other study was designed to confirm the antiulcer activity of *S. persica* decoction using optical microscopy. The elements of gastric mucosa tended to be reestablished normally in treated rats (Almas, 2001).

**Antihyperlipidemic activity**

The effect of prolonged administration of a lyophilized stem decoction of *S. persica* have also been investigated in diet-induced rat hypercholesterolemia. The results showed that the *S. persica* decoction significantly lowered cholesterol and LDL plasma levels in rats (Galati et al, 1997).

**Locomotor activity**

Mice injected with *S. persica* extracts showed significantly low exploratory locomotor activity (Sulaiman et al, 1986).

**Hypoglycemic activity**

Trovato et al. observed significant hypoglycemic activity of *S. persica* in rats (Trovato et al, 1998).
ACE-inhibiting ability
In vitro screening has shows that *S. persica* possesses high ACE-inhibiting ability (Nyman et al, 1998).

Antiplasmodial activity
Nineteen plant species, used traditionally in Sudan against malaria and similar tropical diseases, were evaluated for pharmacological activity by Ali et al. (2002) Different extracts of *S. persica* against *P. falciparum* NF54 strain were found to possess antiplasmodial activity.

Effects on fertility
Darmani et al. (2003) investigated the effects of an extract of miswak for 30 days on the reproductive system of the mouse. The results showed that the exposure to miswak extract did not have much effect on female mouse fertility, although it caused a significant decrease in the relative weights of the ovary and an increase in uterine weights. Exposure of male mice to miswak extract resulted in a 72% reduction in pregnancies in untreated females impregnated by test males. The relative weights of the testes and preputial glands were significantly increased and that of the seminal vesicles was significantly decreased in test males.

Anticonvulsant and sedative potential
The effect of *S. persica* stem extracts on the potentiation of sodium pentobarbital activity and on generalized tonic-clonic seizure produced by pentylenetetrazol (PTZ) on the rats was observed by Monforte et al. (2002). The extracts of *S. persica* extended sleeping time and decreased induction time induced by sodium pentobarbital; in addition it showed protection against pentylenetetrazol-induced convulsion by increasing the latency period and diminishing the death rate.

Cytotoxic activity
The cytotoxic activity of *S. persica* and chlorhexidine (CHX) was evaluated by Rajabalian et al. (2009). The results indicated that both persica and
CHX mouth washes were toxic to macrophage, epithelial, fibroblast, and osteoblast cells in a concentration-dependent manner (Rajabalian et al., 2009).

**Tick-repellent properties**

The *S. persica*, Pistacia, and Juniperus phoenicea were evaluated by Garboui et al. (2009) using host-seeking nymphs of *Ixodes ricinus* in the laboratory for tick-repellent effects of the essential oils. Significant tick-repellent effects were observed for the oils of all three species, but the duration of action was short.
2.3. Tabasheer

Botanical Name: *Bambusa arundinacea*

Family Name: Bambusaceae

Sanskrit Name: Banslochan or Vanshlochan

Common Name (English): Eye of Bamboo

Unani Name: Tabasheer

*Bambusa arundinacea* is a bamboo from Bambusaceae plant family. It grows wild in most parts of tropical India. Stem, young shoots, leaves, seeds and discharge of bamboo"eye" (Tabasheer) is used as a medicinal purpose.

Fig:2.5 *Bambousa arundinacea* Plant

Fig:2.6 Tabasheer
The tall woody bamboo, *Bambousa arundinacea* stems are thorny, numerous, tufted, up to 40 m tall, curving at top and branches numerous, internodes 30–45 cm long, prominent, bearing in lower parts of stems dense half whorls of stiff, naked, horizontal branches, armed with 2–3 recurved, stout spines; lowest nodes rooting; stem-sheaths leathery, orange-yellow when young, hairy outside, shining and ribbed inside, 30–45 cm long; blade triangular, glabrous, covered with a brown felt of bristly hairs inside; leaves thin, linear, up to 20 cm long, glabrous above, hair beneath; leaf-sheaths hairy, small; inflorescence an enormous panicle, often occupying the entire stem; branchlets loose clusters of pale, glabrous spikes.

Tabasheer (also alternative spellings of tabashir and tabaschir) is a siliceous resin found in the joints of the female bamboo *Bambousa arundinacea*. Tabashir is a traditional natural remedy in the south of India and in Bengal. The great interest of Tabashir in phytotherapy is due to its Silica content (97%). Bamboo Tabashir has also been used traditionally as a rich source of naturally occurring organic silica. This synergistic blending of mineral properties makes Silica as one of the most important components of the connective tissue: cartilage, articulation tendon, and some elements of the arterial walls, skin, hair and nails. Besides silica, Tabashir contains iron, calcium, choline and betaine. In general, Tabashir stimulates the natural defense of the organism (during growth, pregnancy, repair of fractures, senescence). This herb is traditionally used to clear obscuration from the heart, mind and lungs. Tabashir an excellent and effective remineralizing agent useful in cases of osteoarthritis, painful joints, fragility of the cartilage (osteoporosis), hair or nails, and in prevention of the consequences of arteriosclerosis. In old age organs responsible for immunity show a deficiency of silica, thus Tabasheer (Banslochan) is helpful in these cases, and also in Alzheimer’s.
The extracts of the plant have been used in Indian folk (traditional) medicine to treat various inflammatory conditions. In Ayurveda leaves, stems and roots are used as astringent, laxative and diuretic. The leaves are sweet, astringent, cooling, emmenagougue, ophthalmic. The plant has got antiulcer activity also. The methanol extract of the leaves of *Bambusa arundinacea* has anti-inflammatory effect and antiulcer activity (Muniappan and Sundararaj, 2003). The extracts of Banshlochan are used along with modern medicines for long-term treatment of chronic inflammatory conditions like rheumatoid arthritis with peptic ulcer. It is also useful in treatment of leprosy, gonorrhea, skin diseases (leukoderma), burning sensation, fever, cough, debilitating (weakening) diseases and in asthma, emphysema, constipating, earache and deafness. An ointment from the root is said to be a folk remedy for cirrhosis and hard tumors, especially tumors of the abdomen, liver, spleen and stomach (Hartwell, 1971). Tabasheer, a siliceous secretion (up to 97% SiO₂), considered aphrodisiac, cooling and tonic and is used in asthma, cough and debilitating diseases i.e. the disease causing weakness (Anonymous CSIR, 1976). Leaves are given to horses suffering coughs and colds. An ethanolic extract of *Bambusa arundinacea* tender shoots (BASE) caused a reduction in fertility of male rats (Vanithakumari et al, 1989).

The people of Hindu religion believe the parsaad offered to the Goddess are nutrients that increase the potential of the brain. The parsaad (food offering) consists of Misri (sugar) nuts, cardammon, nodes of the bamboo shoot (banslochan) and lotus seeds.
2.4 Dementia

Dementia (from Latin de- "apart, away" + mens (genitive mentis)-"mind") causes short and long-term memory impairment and multiple cognitive deficits that compromise social or occupational functioning. Dementia is characterized primarily by a gradual onset of progressive symptoms, including difficulty in learning or retaining information, inability to handle complex tasks, impaired spatial orientation and abilities, language deficits, and behavioral changes (Erkinjuntti et al, 2004).

2.5 Alzheimer's disease

The disease was first identified by Dr. Alois Alzheimer in 1906. Alzheimer’s disease (AD) is the most common cause of progressive mental deterioration; it decreases cognitive function and eventually causes death typically from bronchitis or pneumonia. AD is characterized by multiple cognitive deficits that progress over time and are often accompanied by behavioral disturbances such as aggression, depression and wandering (Carr et al, 1997 Cummings et al, 1998, Cummings and Cole, 2002).

2.6 Stress, cognition and immunity

The health of the immune system depends on the harmonious interaction of all the components that permit the body to identify the presence of abnormal or foreign substances to eliminate damaged and worn out body cells and to destroy abnormal or mutant cells such as cancer (Lucas, 1991).

The concept and definition of a "nootropic drug" was first proposed in 1972 by Guirgea et al (1972) who coined the term "nootropic" from the italic words "noos" (mind) and "tropein" (to turn toward) to mean enhancement of learning and memory and from the Greek words noos or mind and tropos, a bend. Typically, nootropics work by increasing the
brain's supply of neurochemicals (neurotransmitters, enzymes, and hormones), by improving the brain's oxygen supply, or by stimulating nerve growth. Nootropics are drugs used to treat retardation, neural degradation (Alzheimer's and Parkinson's) and for cases of oxygen deficiency to prevent loss of cholinergic neurons which is thought to be responsible for various cognitive deficits (Kornum et al, 2006).

The word 'stress' is defined by the Oxford dictionary as "a state of affair involving demand on physical or mental energy". In medical parlance 'stress' is defined as disturbances of the body homeostasis (Ramsey, 1982). The constant stress conditions result in a loss in neural and hormonal balance. When body fail to counter a stress situation as results in stress related physical symptoms such as tense muscles, unfocused anxiety, dizziness and rapid heart beats (Graham et al, 2010, Chrousos and Gold, 1992). Stress can increase the risk of both acute and chronic psychosomatic illnesses and weaken the immune system of the human body. Stress can cause headaches, irritable bowel syndrome, eating disorder, allergies, insomnia, backaches, frequent cold and fatigue to diseases such as hypertension, asthma, diabetes, heart ailments and cancer (McCormick et al, 2008, Padgett and Sheridan, 2006, Buwalda et al, 2005).

The immune system and the brain are linked together through signaling pathways. The brain and the immune system are the two major adaptive systems of the body. This process is essential for maintaining homeostasis. Two major pathway systems are involved in this cross-talk: the hypothalamic-pituitary-adrenal axis (HPA axis) and the sympathetic nervous system (SNS). The activation of SNS during an immune response might be aimed to localize the inflammatory response. The body's primary stress management system is the HPA axis. The HPA axis responds to physical and mental challenges to maintain homeostasis in
part by controlling the body's cortisol level. Dysregulation of the HPA axis is implicated in numerous stress-related diseases. HPA axis activity and cytokines are intrinsically interconnected: inflammatory cytokines stimulate adrenocorticotropic hormone (ACTH) and cortisol secretion. In turn, glucocorticoids suppress the synthesis of proinflammatory cytokines. Cytokines are also locally produced in the brain, especially in the hypothalamus contributing to the development of behavioral effects (Covelli et al., 2005), in the hippocampus interfere with the consolidation of memory and within the spinal cord exaggerate pain (Maier and Watkins, 2003).

Stress is thought to affect immune function through emotional and/or behavioral manifestations such as anxiety, fear, tension, cognition, anger and sadness and physiological changes heart rate, blood pressure and sweating. Researchers have suggested that these changes are beneficial if they are of limited duration (Chrousos and Gold, 1992). In chronic stress, the system is unable to maintain equilibrium or homeostasis. Immune function is observed impaired in healthy people who are experiencing stress (Zorrilla et al., 2001). Naturalistic stress conditions are associated with increase in number of circulating neutrophils, decreases in number and percentages of total T-cells and helper T-cells, decrease in percentages of Natural killer cell (NK) cells and cytotoxic T-cell lymphocytes. Hans Selye experimented with animals putting them under different physical and mental adverse conditions and noted that under these conditions the body consistently adapted to heal and recover like enlargement of the adrenal gland, atrophy of the thymus, spleen and other lymphoid tissue, and gastric ulcerations (Neylan, 1998).

Chronic secretion of stress hormones, glucocorticoids (GCs) and catecholamines (CAs) as a result of disease, may reduce the affect of neurotransmitters, including serotonin, norepinephrine and dopamine, or
number of reception in the brain, thereby leading to the dysregulation of neurohormones. After stimulation, norepinephrine is released from the sympathetic nerve terminals in organs and the target immune cells express adrenoreceptors. Through stimulation of these receptors, locally released norepinephrine or circulating catecholamines such as epinephrine affect lymphocyte traffic and circulation, proliferation and modulate cytokine production and the functional activity of different lymphoid cells. The systemic or neuro-inflammation and neuroimmune activation have been shown to play a role in the etiology of a variety of neurodegenerative disorders such as Parkinson's and Alzheimer's disease, multiple sclerosis, pain and AIDS-associated dementia. Glucocorticoids also facilitate the glycogenolysis, gluconeogenesis in the liver. Stressors can produce profound health consequences. In one epidemiological study, for example, all-cause mortality increased in the month following a severe stressor – the death of a spouse (Kaprio et al, 1987). Theorists proposed that stressful events trigger cognitive and affective responses which, in turn, induced sympathetic nervous system and endocrine changes, which ultimately impaired immune function (Chrousos and Gold, 1992).

Circulating glucose concentration regulate many brain functions including learning and memory (Korol and Gold, 1998). A recent report by Kaplan et al (2000) suggested that glucose enhances cognitive performance. This work is supported by extensive evidence that modest increase in circulating glucose concentration enhances the formation of new memories in rodents and human. Glucose enhances memory for several different tasks in rodents. In human, glucose enhances memory in healthy young and elderly persons and in persons with Alzheimer disease or Down syndrome (Ragozzino et al, 1996). The glucose is the major source of energy for the central nervous system. This suggestion is
supported by the observation that microinjections of glucose into the septohippocampal system of rats enhances cognitive functioning (Ragozzino et al, 1992) or memory impairments that can be reversed by administration of epinephrine or glucose in rodents (Korol and Gold, 1998). In this context, it is interesting to note that glucose is critical for the production of acetyl-CoA, a precursor of acetylcholine (Tucek and Cheng, 1974) and epinephrine enhances the glycogenolysis as well as gluconeogenesis, and that decrease in glucose concentration results in decrease in brain acetylcholine (Gibson and Blass, 1976). Thus, one strong possibility is that glucose enhances memory processes by increasing acetylcholine synthesis and release (Ragozzino et al., 1996). This is substantiated by the observation that glucose can modify the effects of cholinergic drugs on various behavioral and neural measures. Furthermore, extracellular brain glucose concentration varies with neuronal activity indicating that glucose may be critical in modulating memory function (Gibson and Blass, 1976). This is supported by the report that hippocampal acetylcholine release is increased in rats during a spatial task (Ragozzino et al, 1996). Insulin receptors are present in brain cells and may play a role in brain cognitive functions (Zhao et al, 1999), including learning and memory. The hyperinsulinemia also improves memory in patients with Alzheimer disease (Craft et al, 1996).

Neutrophils comprise the body’s first line of white blood cell immune defense - they are normally first to arrive at wound/injury sites. Yet our modern sugar-rich diet has been shown in multiple studies to significantly impair neutrophil activity (i.e. enhances neutrophil self-destruction) (Huemer and Challem, 1997).
2.7 Animal models for assessing cognitive behavior
Most of the animal models for cognitive functions can be discussed under two behavioral tasks.

1. Behavior on maze (such as elevated plus maze, radial arm maze, T-maze, Y-maze, figure eight maze and water maze).
2. Behavior on avoidance chamber (include active and passive paradigm).

2.7.1 Avoidance behavior test
An avoidance paradigm consists of a box (shuttle box) that is divided into two compartments by a central wall. This wall has an opening through which the animal can pass between two compartments. Each compartment is equipped with buzzer alarm and a light source. Animal receives electric shock through a shocker grids span over the floor of the box. Animal must relocate into adjacent compartments response to shock. These shocks are buzzer (conditional or unconditional) stimuli, induces active avoidance to the animal. The animal must relocate the compartment in order to avoid mild electric shock within a present time (Cahill et al, 1986; Pereira et al, 1989). The transfer latency of the animal is noted and is related to the retention of the memory task.

In passive avoidance test, animal is placed on a shock free zone of a light box with an electrifiable grid floor. When animal step off the shock free zone, it receives a constant and continuous electric shock through the grid floor. The normal reaction of the animal is to jump back on to the platform. Failure or latency to transfer to a shock free area indicates the passive avoidance behavior which is used as a short term memory task (Sharma and Kulkarni, 1992; Isaacson et al, 1995). Step down latency is noted after training and after drug treatment. The test ended when the animal refrain from stepping down for atleast 60 seconds.
2.7.2 **Plus maze** (Itoh et al, 1990)

It consists of a central platform from which radiated symmetrical arms. The animal (rats/mice) is placed on the central platform and transfer latency is noted. Amnesic drug treatment prolongs the transfer latency while nootropic drugs shorten it. The transfer latency is the time in the animal to move from the open arms to the enclosed arms. It is used to measure the long-term memory.

In addition maze can be used to evaluate spontaneous alternation behavior (SAB) by measuring percentage alternation (Raggozino et al, 1998). Nootropic drugs increase the percentage alternation whereas amnesia drugs reduce it.

2.7.3 **T–Maze**

This is used to assess the spatial and long-term memory. At the extremity of each arm, a food cup is located on the floor. The animals are familiarized with the maze, food and food containers. Animals are food deprived for 24 hours and then trained with only one arm of T-maze which is baited with food. A correct trail ended with the animal eating the food. An incorrect trial ended with the animal reaching the empty food cup.

2.7.4 **Y-maze**

Each animal is placed at the end of one arm and allowed to move freely for eight minutes. Animal tends to explore the maze systematically, entering each arm in turn.

The percentage alternation is calculated according to the method of Starter et al (1988)

\[
\text{Actual number of alternations} \times 100 \\
\frac{\text{Number of arm entries} - 4}
\]
Y- Maze can be used to assess spatial and long-term memory (Starter et al, 1988).

2.7.6 **Three panel runaway apparatus** (Furuya et al, 1988)
This apparatus is composed of a start box, a goal box and four consecutive choice points. Each choice point consists of three panel gates (A, B and C). The animals are trained to reaches the goal box with the correct panel gate position for each animal being changed everyday but not in each session. The time required for the animal to take pellet (latency) and the number of pushing incorrect gates (number of errors) are noted. Error responses reduce gradually with training. The effect of drug is noted on these two parameters. This method is used for assessing working in rats.

2.7.7 **Morris water maze** (Morris, 1984)
This method is a more advanced technique to assess the memory, which is used to evaluate spatial memory of the animal. In this, rats or mice are placed into a circular pool of water body contained a hidden platform. Assessment is done by testing the latency of the animal to escape from the water by climbing onto the hidden platform. Training reduces latency period significantly. The path length, direction and path taken can be recorded by an automated video system. Other models to assess memory include electronic induced amnesia, brain lesion induced cognitive dysfunction and drug induced state dependence learning.

2.8 **Animal stress models commonly used**
Animal models of stress that use physical stress and psychological stress can be given as follows (Bhatia et al, 2011)

1. Temperature fluctuation induced stress:
   a. Immersion in cold water with no escape
   b. Cold environment isolation
2. Immobilization induced stress
3. Electric foot shock induced stress
4. Forced swimming induced stress
5. Neonatal isolation induced stress
6. Predatory stress
7. Day-night light change induced stress

2.8.1 Forced swimming induced stress model

It is the tendency of the living being to escape or avoid a noxious stimuli/condition. If the animal is not able to escape the stressful stimuli or it feels threatened, the animal will show stress response. This principle is used for developing forced swimming model for inducing stress in laboratory animals. In order to produce swimming induced stress, rats are made to swim in a cylinder (30cm diameter and filled to a height of 20cm with 15cm of space above the head of the rat) for a single session of 2h duration for acute stress, or for one 2h session a day for five consecutive days for chronic stress (Ferry et al, 1991). Some authors have used forced swimming in warm (20°C) water for 3 minutes with the total session lasting for 1h. Although forced swimming induced stress is a highly safe model, adaptation to chronic swimming induced stress has been reported and inter-strain differences between rats to forced swimming behavior have also been documented.