CHAPTER-I

INTRODUCTION
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1. Introduction:

Today stress is a constant reality and has become part of the modern lifestyle. The struggleful life style of people subjected to tremendous physical exercise and mental tension gives stress and strain to a person living in modern society, concomitantly increasing the needs of life and changing fashion of living pattern under the unsafe environment. Stress is probably the most powerful influence on human being next to genetics. It exerts profound effects on physical, physiological and mental status, which disturb the equilibrium of the human body, resulting in loss of energy, vigor, vitality, immunity and stamina with every day consistency. Research has been on going for decades to deconstruct the effect of stress on human body and find out compounds that will reduce its negative effects. Amongst the different stresses “oxidative stress” caused by reactive oxygen species (ROS) like super oxide, singlet oxygen, peroxynitrite or hydrogen peroxide, shifts the balance in favour of peroxidation. Oxidative stress has been implicated in aging and age related disorders (Sies, 1986).

ROS is the generic term given to a group of molecules formed when unpaired electrons from hydrogen, oxygen and number of transition metal ion react to form highly reactive species. ROS originate from different enzyme systems within the cell including the NADPH oxidise system (Brown and Griendling, 2009). ROS can damage many biological molecules including DNA, lipids, proteins and causes modification of these biomolecules which disrupts their normal function(s) and can lead to cell death. So it has been well established that ROS have a central role to play in the induction of apoptosis (Sorcha and Thomas, 2009). Uncontrolled accumulation of ROS often leads to oxidative stress; causing cellular damage is often triggering for the initiations of apoptosis. Under normal physiological conditions the redox status of a cell is
maintained between antioxidants and ROS in order to prevent such oxidative stress.

Most of the antioxidant herbs are promising candidates in treatment of oxidative stress. Number of the Indian medicinal plants has proven antioxidant potential activities. Although, so many medicinal plants used in India’s “Ayurveda”, but the most important is *Withania somnifera* (Ashwagandha) due to its wonderful activities. Ashwagandha is a herb that improves the body’s ability to maintain physical efforts and help the body to adopt various types of stress. It is India’s most potent hot plant. Many western herbalists refer to this herb as “Ayurvedic ginseng” because of its reputation for increasing energy, strength, stamina and its ability to relieve stress. It is also known as “Indian ginseng”. It is a supreme important medicinal herb in the Ayurvedic and Indigenous medicinal system for over 3000 years (Unger, 2007). Clinical studies of Ashwagandha in various universities and research institute have demonstrated that, it increases resistance to greater physical endurance and more ability to withstand stress (Grandhi et al, 1994).

2. **Botanical Aspect of Ashwagandha:**

A. **Classification:**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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<tr>
<td>Subkingdom</td>
<td>Tracheobionta</td>
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<tr>
<td>Division</td>
<td>Magnoliophyta</td>
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<tr>
<td>Class</td>
<td>Magnoliopsida</td>
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<tr>
<td>Subclass</td>
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<tr>
<td>Genus</td>
<td>Withania</td>
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<tr>
<td>Species</td>
<td>somnifera.</td>
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<tr>
<td>Scientific name</td>
<td><em>W. somnifera</em> (L.) Dunal</td>
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The generic name *Withania* is supposed to be in honor of H. Withan, a British geologist and writer of fossil botany of the nineteenth century.

The genus represented by two species in India viz. *Withania coagulens* Dunal, is restricted to Sindh while *W. somnifera* is widely distributed throughout the drier subtropical region of India, ascending to 1800 meters in Himalaya.

Ashwagandha is recognized by various local names viz. Ashwagandha, Winter cherry, Ashgandha, Achuvagandi, Amikkiragaddy, Amukran-Kizhangy, Asagandha, Asana, Asgandh, Asundha, Asvagandhi, Hirimaddina-gaddy, Hirreguddy, Penneroa-gadda, Pevette, Sogudeberu, Dhorgunj, Ghoda, Tilli, Askandha, Askand, etc.

**B. Distribution:**

It is naturally found in diverse area, ranging from the Africa, the Mediterranean (Israel), Canary Islands and East India. It is also found throughout the drier parts of India, into West Asia and Northern Africa (Warrier *et al*, 1996).

It is native to India, Pakistan and Shri Lanka but also found in Bangladesh, Australia, East Asia and Africa. In India it is distributed in drier parts up to 5,500 m. It is found in western India, Maharashtra, Gujarat, Rajasthan, Madhya Pradesh, Tamil Nadu, Karnataka, Bengal and extends to the mountain regions to Himachal Pradesh. Plants are restricted to places around human dwellings and road sides, where there is accumulation of domestic wastes and the land rich in organic carbon content.

**C. Morphology:**

Ashwagandha is a small, branched, evergreen perennial and woody shrub grows usually up to two feet in height. Because of its wide range of
occurrence, there are considerable morphological and chemical variations in terms of local species.

**Leaves:** The plant is covered by the leaves all round the year. Leaves are ovate, entire, sub acute, stelletely pubescent, base acute, conspicuous and petioles up to 2 cm. long.

**Flowers:** Ashwagandha has sessile, axillary, greenish or lurid yellow and hermaphrodite flowers. Pedicel is short.

**Fruits:** The fruit is orange red, berry and smooth oblong, rounded or somewhat produced at base.

**Seeds:** The seeds are yellow and scurfy.

**Roots:** The roots are more or less tuberose, whitish brown in color. These are fleshy, cylindrical, epidermis is light brown and medulla is white (Yadav, 1983).

**D. Constituents:**

The chemistry of *W. somnifera* has been extensively studied and over 35 chemical constituents have been identified, extracted and isolated (Rastogi and Mehrotra, 1998). The biologically active chemical constituents are alkaloids (isopelletierine, anaferine) steroidal lactones (withanolides, withaferins) saponins containing an additional acyl group (sitoindoside VII & VIII) and withanolides with a glucose at carbon 27 (sitoindoside IX & X). *W. somnifera* is also rich in iron.

The distinctive earthy odor and flavor of ashwagandha is due to presence of certain steroidal lactones or withanolides (Schwarting, 1963 and Bhatnagar, 1976).

Ashwagandha’s chemical soul is “withanolides” a novel compound used to standardize the potency of extracts. To date up to 19 withanolides derivatives have been isolated from *Withania* roots (Padmavati et al, 2005). Ashwagandha’s steroidal compound are of great interest to researchers, including ergostane type steroidal lactones, including
withanolides A – Y, dehydrowithanolide – R, Withasomniferin – A, Withanone and others. Other constituents include the phytosterols sitoindoside VII – X and bi–sitosterol as well as alkaloids (e.g. Ashwagandhine, cuscohygrine, tropine, pseudotropine, isopelletierine and anaferine), a variety of amino acids including tryptophan and high amount of iron (Williamson, 2002).

These molecules which are steroidal in nature and are believed to account for the multiple medicinal applications of ashwagandha, among these different constituents glycowithanolides is the active principle of *W. somnifera* (Bhattacharya *et al*, 1997). Glycowithanolides is the major bioactive chemical principle of *W. somnifera* (Ghosal *et al*, 1989).

3. **Uses:**

Long ago before the birth of Buddhism, Yoga and Christianity, there was recorded the use of Ashwagandha as healing herb. Today after thousands of years of continuous use, it is recorded one of the most valuable Ayurvedic medical plant. Many pharmacological studies have been conducted to investigate the properties of Ashwagandha in an attempt to authenticate its use as a multipurpose medicinal agent (Dhuley, 2000). Ashwagandha is an important medicinal plant widely used as a home remedy for several diseases in India as well as other parts of the world (Owais *et al*, 2005).

- Ashwagandha is used against general debility and increase longevity (Gupta *et al*, 2003) as well as sexual debility. (Moose, 1976; Lewis and Elvin, 1977).
- It is used as an adaptogen (Bhattcharya, 1993; Grandhi *et al*, 1994).
- It is used as sleep inducing agent, tranquilizer, and nurture the nervous system.
- Used against rheumatism (Chopra *et al*, 1965)
- Used against digestive complaints (Grandhi *et al*, 1994).
• To cure ulcer, carbuncles, eye sore (Warrier, 1996).
• Used against respiratory problems (Kirtikar and Basu, 1935; Etheresia et al, 2009).
• Used against reproductive problems (Nadakarni, 1954; Moose, 1976; Sharma, 2002).
• Used as anticancer drug (Stan et al, 2008)
• Used as an antioxidant (Bhattacharya et al, 2001)
• As an immunity enhancer (Prabhakar et al, 1994; Uniyal, 2002).
• As an antiaging (Kuppurajan et al, 1980; Kulkarni, 1991; Satyvati, 1995).
• Used as kidney tonic.
• Used as an antibiotic (Kirtikar and Basu, 1933; Levis and Elvin-levis, 1977).

4. National and International Research On Ashwagandha:

A. Antistressor effect of Ashwagandha:

Ashwagandha is widely used in Ayurvedic and western herbal formulas as an adaptogen – a substance that can help our body naturally adapt to physical, emotional and environmental stresses. Ashwagandha is reported to have tonic or adaptogenic effect similar to Panax ginseng (Grandhi et al, 1994). The herbal remedies referred to as “ginseng. Panax ginseng is one of the commonly used ginseng in research. It also called as Asian ginseng or Korean ginseng. Main active component of Panax ginseng is ginsenosides which has been shown to have a variety of beneficial effects, including anti-inflammatory, antioxidant and anticancer. It may improve psychological function, immune function and condition associated with diabetes (David and Tracipantuso, 2003). Ginseng is one of the most well known herbal medicines widely used in
East Asia as a tonic, restorative and antiaging agent in traditional Chinese medicine (Kim et al, 2002; Kaufman et al, 2002; Xie et al, 2006).

Ashwagandha has been shown to increase stress resistance so called “antistressor”. Various studies on animals have been performed to investigate its use as an antistressor. Singh et al, (1982) evaluate anti stress effect of *W. somnifera* by giving alcoholic extract from seeds of it to mice before subjecting to swimming test and found doubled the swimming time as compared to the control. Archana and Namasivayam, (1999) found better stress tolerance in rats treated with *W. somnifera* extract and subjected to cold water swimming test. Alcoholic extract of *W. somnifera* reduced blood urea, nitrogen level, blood lactic acid and adrenal hypertrophy in rats subjected to stress (Dadkar et al, 1987). Bhattacharya et al, (2003) in comparative study of Ashwagandha and benzodiazepine in mice showed that Ashwagandha exhibited strong antidepressive effect, hence it can be used as an anti stress adaptogen. Stress reducing effects of Ashwagandha and Panax ginseng in rats was studied by Bhattacharya et al (2003). Ramrao et al, (2006) reported that pre treatment of *W. somnifera* in Swiss mice develop tolerance to morphine induced GIT inhibition and analgesia. Suggesting *W. somnifera’s* potential in alleviating the adverse effects of morphine and attendant immuno – depression.

B. Anti inflammatory effect of Ashwagandha:

Anti inflammatory properties of Ashwagandha have been investigated to validate its use in inflammatory arthritis (Anbalagan et al, 1981, 1984; Somasundaram et al, 1983). Begum and Sadique (1988) examined the effect of *W. somnifera* root powder on paw swelling and bony degenerative changes in Freud’s adjuvant – induced arthritis in rat and found significant reduction in both. Rasool and Palaninathan, (2006) studied suppressive effect of *W. somnifera* root powder on experimental
gouty arthritis in monosodium – urate – crystal induced rats and concluded that *W. somnifera* is potent analgesic and antipyretic without causing any gastric damage.


C. *Anticancer / antitumour effect of Ashwagandha:*

Natural compounds have shown promising outcomes in cancer therapy for the past 30 years. Bioactive constituent isolated from Ashwagandha – withaferin has shown antitumour activity (Handa and Kapoor, 2003). Introduction of withanolides in the diet may prevent or decrease the growth of tumors in human (Jayaprakasan et al, 2003). Anon, (2004) reported that Withaferin – a specific compound extracted from Ashwagandha was more effective than doxorubicin in inhibiting breast and colon cancer cell growth. Therapeutic potential of characteristics phytochemical form an important facet of anticancer drug discovery because medicinal plants have very long history of safe consumption and bioactive compounds obtained from them are normally nontoxic or less toxic to human (Newman et al, 2000). Diwanay et al, (2004) reported that total extract of *W. somnifera* and *Tinospora cardifolia* (80:20) as well as alkaloid free polar fractions of *W. somnifera* resulted in protection towards cyclophosphamide treated mouse ascitic sarcoma. They also reported increase in white blood cells count and hemagglutination and hemolytic antibody titer. Thus these drugs will be important in development of supportive treatment with cancer chemotherapy. Christina et al, (2004) evaluated the effect of ethanolic root extract of *W. somnifera* (REWS) against Dalton’s Ascitic lymphoma in Swiss albino mice and found a significant increase in the life span, decrease in a cancer cell number as well as tumor weight in the tumor induced mice. Padmavathi et al, (2005) reported that orally
administered Ashwagandha extract significantly inhibited experimentally induced stomach cancer in Swiss albino mouse. Palaniyandi et al, (2006) evaluated the therapeutic effect of *W. somnifera* along with paclitaxel on lung tumor induced by benzopyren in male Swiss albino mice and found that the activities of different ATPase were reversed to near normal control values in animals treated with *W. somnifera*. Thus *W. somnifera* inhibits free-radical mediated cellular damage and decreased lipid peroxidation. Mathur et al, (2006) found that Ashwagandha extract disrupts cancer cell’s ability to reproduce and also possesses anti-angiogenic activity. Hinge et al, (2007) demonstrated that tumor proteasome B₅ subunit is primary target of Withaferin-A and inhibition of the proteasomal chymotrypsin – like activity by Withaferin-A in *vivo* is responsible for or contributes to the antitumour effect. Panjabmurthy et al, (2008) indicated that withaferin – A significantly inhibited abnormal cell proliferation and differentiation during 7-12-Di-methyl-benzaanthracene (DMBA) induced oral carcinogenesis in male golden Syrian hamsters. Mulabagal et al, (2009) investigated a novel withanolides sulfoxide compound from roots of *W. somnifera* and have ability to inhibit cyclo-oxygenase – 2 – enzyme and suppress human tumor cell proliferation.

**D. Effect of Ashwagandha on body weight:**

Venkatraghavan et al, (1980) reported growth promoting effect of *W. somnifera* in healthy 8-12 years aged children and found increase in body weight in children received purified powder of *W. somnifera* than the control group. Sharma et al, (1986) monitored body weight, body temperature, general toxicity, well being, number of pregnancy, litter size and progeny weight of rats administered with *W. somnifera* and found that *W. somnifera* treated groups gained more body weight than control group. Aphale et al, (1998) observed significant increase in the body

E. Effect of Ashwagandha on Nervous System:

Ghosal et al, (1989) evaluated effect of glycowithanolides and a mixture of sitoindoside IX & X isolated from W. somnifera on central nervous system. These compounds produced significant anti stress activity in albino mice and rats and augmented learning acquisition as well as memory retention in young and old rats. Total alkaloid extract of W. somnifera roots has been studied for its effects on central nervous system (Malhotra et al, 1981). He found that W. somnifera exhibited a taming effect and a mild depressant (tranquilizer) effect on nervous system in monkey, cats, albino rats and mice. Bhattacharya et al, (1997) reported that the major bioactive chemical of W. somnifera appears to have significant effect in various areas of rat brain. W. somnifera has also been shown to be effective in alleviating several central nervous system disorders like epilepsy, anxiety, depression, catalepsy, morphine tolerance, tardive. Bhattacharya et al, (2000); Jain et al, (2001); Dhuley, (2001); Ahmad et al, (2005); Kumar et al, (2006) and Naidu et al, (2006) evaluated anti-parkinsonian effect of W. somnifera extract on 6-hydroxydopamine induced Parkinson rats and reported that W. somnifera has
potent antioxidant, antiperoxidative and free radical quenching properties in various diseased conditions, which might be helpful in protecting neuronal injury in Parkinson’s diseases. Elsakka et al, (1990) reported that Ashwagandha is anabolic, containing substantial amount of arginine and arnithine which are needed for proper nervous system functions. Schliebs et al, (1997) reported the cognition enhancing and memory improving effect of *W. somnifera* extract in animals and in humans. Tohda et al, (2000) found that Ashwagandha help support the growth of nerve cell dendrites which allow these cells to receive communications from other cells. So Ashwagandha could help to heal the brain tissue changes that accompany dementia. Kuboyama et al, (2002) found that six of the 18 compounds isolated from methanol extract of Ashwagandha enhanced neurite out growth in human neuroblastoma SH-SYSY cells. Axons are predominantly extended by withanolide-A and dendrites by withanolides IV & VI. Kuboyama et al, (2005) noted that Ashwagandha supported significant regeneration of axons and dendrites of nerve cells, reconstruction of synapses in validated model of damaged nerve cells and impaired nerve signaling pathway, suggesting that Ashwagandha is a potent natural drug for treating neurodegenerative diseases like Alzheimer’s. Ashwagandha help to promote the growth of both normal and damaged nerve cells, hence it may boost healthy brain cell function as well as benefit diseased nerve cells (Tohda et al, 2005).

F. Immunity enhancing effect of Ashwagandha:

Ghosal et al, (1989) evaluated immunomodulatory effects of glycowithanolides and a mixture of sitandosides IX & X isolated from *W. somnifera* in Swiss mice and Wister stain albino rats and found that these compounds produce activation of peritoneal macrophages, phagocytosis and increased activity of the lysosomal enzymes. Arora et al, (2004) reported methanol and hexane extract of leaves and roots of
Ashwagandha have potent antibacterial activity. Ashwagandha has been shown to increase non-specific general immunity in children (Prabhakar et al, 1994). Uniyal, (2002) in various test studies found that withanolides in Ashwagandha possess immuno-suppression and immunostimulatory properties that activate immune responses. Iuvone and Esposito, (2003) while elucidating the mechanism of *W. somnifera* for its immunostimulating effect found that the methanolic extract of Ashwagandha root increase nitric oxide (NO) production by macrophages indicating the immunostimulant properties of Ashwagandha. Ziauddin et al, (1996) studied immunomodulatory effect of Ashwagandha in mice and found significant increase in hemoglobin concentration, red blood cell count and platelet count in Ashwagandha treated mice. Davis and Kuttan, (2000) found increased total WBC count, bone marrow cellularity, enhancement in the circulating antibody titer and enhancement in phagocytic activity of peritoneal macrophages in *W. somnifera* treated Balb/c mice. Owais et al, (2005) evaluated antibacterial activity of both aqueous as well as methanolic extract of *W. somnifera* (L.) Dunal roots and leaves against experimental salmonellosis in Balb / c mice and found increased survival rate as well as less bacterial load in various vital organs of the treated murine.

**G. Effect of Ashwagandha on Endocrine system:**

*W. somnifera* along with *Tinospora cardifolia, Eclipta alba, Ocimum sanctum, Picorrhiza kurroa* and Shilajit caused a dose related decrease in streptozotocin induced hyperglycemia (Ghosal et al, 1989). Panda and Kar, (1998) observed that *W. somnifera* root extract in mice significantly increased the serum levels of 3, 3’- 5 – tri-iodothyronine (*T_3*) and tetraiodothyronine (*T_4*), suggested that *W. somnifera* stimulates thyroid gland activity. Anwer et al, (2008) reported that aqueous extract
of *W. somnifera* normalizes hyperglycemia in diabetes mellitus rats by improving insulin sensitivity.

5. **Cell Secretion:**

A substance that is synthesized in the cell especially in a glandular cell and released from it is called as cell secretion. Secretion can be produced by a single cell or by a group of cells called a gland. Some secretions perform a special functions in the body are called true secretions and others are eliminated as waste products called excretions.

Digestive secretion includes saliva, gastric juice, intestinal juice, pancreatic juice and bile juice. Certain secretions serve as lubricants e.g. synovial fluid in joints or secretions from mucous membranes from the lacrimal glands. The mammary glands secrete milk. The endocrine gland secretes hormones that enter directly in the blood stream. Among the excretions from the body are urine (from kidneys), perspiration (from the sweat glands) and bile pigments (from the gall bladder). Both the exocrine and endocrine glands are involved in cell secretion. Lacrimal glands, mucus glands, nasal glands, sweat glands, liver, salivary glands, etc. are the exocrine glands involved in the cell secretion.

6. **Structure and Functions of Salivary Gland:**

A. **Structure of salivary glands:**

Salivary glands are ectodermal in origin and are exocrine glands, secrete saliva. There are three pairs of major salivary gland viz. sub-mandibular gland, sub-lingual gland and parotid gland, present outside the oral cavity in most of the mammals and are provided with long duct system. These glands are different in their structure and location. The secretion of these glands is called saliva.

The glandular cells are of two kinds viz. serous and mucous. Depending upon the predominance of one or the other cell type salivary glands are divided into serous, mucous and mixed serous and mucous.
Each gland consists of parenchymal and stromal components. Parenchymal components include secretory acini, tubules and duct system. While stromal component includes supporting framework of fibrous connective tissue consisting of blood vessels, nerve fibers, lymphatic vessels.

The glandular cells are arranged into tubes, acini and ducts. The secreting acini of the salivary glands are composed of pyramidal cells of a simple columnar epithelium rest on basement lamina. Between the pyramidal cells and basement lamina are a few myoepithelial cells. The duct system of salivary glands is composed of several differently structured segments, the cells of which exerts an influence on the ionic concentration of the primary fluid during its passage to the oral cavity through intercalated ducts, striated ducts (secretory ducts) and interlobular (excretory) ducts.

- **Excretory ducts:**
  
  These ducts carry the secretion towards mouth cavity. The main excretory duct in each instance is lined by stratified squamous epithelium at the point of its opening into mouth. Elsewhere it is formed by two layers of stratified columnar epithelium or pseudostratified epithelium.

- **Striated (secretory) Duct:**
  
  Smaller subdivisions of the excretory ducts enter the lobules of glands and divided further in to secretory ducts. The secretory ducts are formed of simple columnar epithelium. The cells appear striated indicative of some secretory activity.

- **Intercalated ducts:**
  
  The secretory ducts branch into much smaller intercalated ducts, made up of flattened or low columnar epithelial cells. These in turn open into the secreting lobules or acini.
a. **Submandibular glands (SM):**

These are tubulo-alveolar paired glands, lying on either side of the midline of body, behind the mandible in the floor of the mouth in the ventral cervical region. Externally they are covered by connective tissue capsule that sends many septa inside and dividing the gland into number of lobes and lobules. Their end pieces are made up of more or less spherical mass of cell called acini. In addition they consist of granular convoluted tubules (GCT) and duct system, made up of intercalated ducts, striated ducts and excretory ducts. The intercalated ducts are shorter, narrow and possess secretory activity similar to the acinar cells. The striated ducts are longer and more numerous than parotid which composed of tall pyramidal cells. The stromal component of gland includes the spongy connective tissue containing blood vessels, the capillaries of carotid arteries, nerve fibers arising from sympathetic and parasympathetic components of autonomous nervous system, which regulate the synthesis, secretion of saliva and its chemical concentrations as well as its secretion in mouth. Acinar cells together form acini, they are pyramidal in shape with basally placed spherical nucleus. In between pyramidal cells and basal lamina present numerous myoepithelial cells. Acinar cells are seromucous and secrete mainly glycoproteins (Spicer *et al*, 1964). Granular convoluted tubules have long been interested because these are considered to be a site of formation of many enzymes like kallikrein (Orastavik *et al*, 1975; Hojima *et al*, 1977), proteases (Bhoola *et al*, 1973), nerve growth factor (Goldstein and Budman, 1965; Ellison, 1967), epidermal growth factor (Cohen, 1962; Young and Van Lennep, 1978) and various mesodermal growth factors (Weimer and Haraguchi, 1975).
b. **Sublingual salivary glands:**

These are paired glands and without capsule. The general structure of these glands is acini, intercalated ducts and striated ducts. Acini are formed of mucous gland cells, which are pyramidal in shape, with basally located nucleus. The mucous acini (MA), frequently capped with serous demilunes (DM). Demilunes are the half-moon shaped structure made up of demilunar cells and are intimately attached to the mucous acini. Duct system is poorly developed in sublingual glands, (Young and Van Lennep, 1978) it is shorter and simpler than parotid glands. The intercalated ducts are rarely seen. The secretion granules are absent in intercalated duct. The secretory ducts are directly continuous with tubules and acini.

c. **Parotid glands:**

These are the paired glands located in front of the ear, folded around each ramus of the mandible and are covered by fibrous connective tissue capsule. These are exclusively serous glands, consists of acini which have pyramidle shaped secretory cells. Duct system is well developed consisting of intercalated duct and striated duct (Young and Van Lennep, 1978).

**B. Functions of salivary glands:**

Salivary glands secrete saliva, various enzymes, hormones, pharmacologically active components and glycoproteins.

a. Saliva behaves as buffer system to protect the mouth from acidogenic micro-organisms and preventing enamel de-mineralization (Nagler, 2004).

b. Calcium and phosphate ions in saliva bring re-mineralization of carious tooth (Stack and Papas 2001).

c. Due to presence of $\alpha$ - amylase in saliva, it brings about digestion of starch and favoring the formation of the food bolus.
d. Due to presence of mucin in saliva, saliva forms a seromucosal covering that lubricates and protects the oral tissue against irritating agent (Stack and Papas, 2001).

e. Mastication, speech and deglutition are aided by lubricant effect of mucin (Humphrey and Williamson, 2002; Amerongan and Veerman, 2002; Nagler, 2004).

f. Mucin provides protection against dehydration and maintenance of salivary visco-elasticity.

g. Mucin protects the oral tissue against micro-organisms attack.

h. Secretory immunoglobulin-A (Ig-A) in saliva can neutralize viruses, bacteria and enzyme-toxins.

i. Saliva represents an increasingly useful auxiliary means of diagnosis, it is an indicator of risk for diseases creating a close relation between oral and systemic health (de-Almeida et al, 2008). Diminished functions of salivary glands leads to various old age related diseases such as xerostomia, dental caries, and periodontal diseases. Salivary gland biopsy can be used to diagnose conditions such as Sjogren’s syndrome (Olver et al, 2006).

j. Protein constituents of saliva have number of biological functions that are intimately involved in maintenance of oral health. Several biologically active polypeptides such as Epidermal Growth Factor (EGF), Nerve Growth Factor (NGF), Transforming Growth Factor (TGF), Fibroblast Growth Factor (FGF), and Insulin – like – Growth factor (IGF-1) are found in submandibular glands (Sporn et al, 1982) and were reported to accelerate the rate of wound healing in mice and human (Brown et al, 1989)

k. Due to presence of Epidermal Growth factor in submandibular gland helps for tissue repairing. (Schultz et al, 1991).
1. Salivary glands play important role in differentiation, development of testis and associated organs (Bodare and Pillai, 2007; Walvekar and Pillai, 2008).

m. IGF-1 from submandibular glands plays important role in stimulation of DNA synthesis, aminoacid uptake, protein synthesis and glucose transport (Melvin, 1993)

n. Saliva contains lysozymes, peroxidases and immunoglobulins (Young and Van Lennep, 1978) have antibacterial and antiviral properties.

o. Glycoproteins in saliva have a lubricating property and help the tongue, oral mucosa and teeth to function smoothly in speaking and swallowing.

7. Changes in Salivary Glands During Aging:

A. Structural changes:

included oncocytic proliferation, fatty infiltration, squamous and mucous metaplasia, hyperplasia and atrophy, followed by degeneration of the parenchymal tissues was found in wistar rats (Warren, 1949 a; Scott, 1977a; Martinez and Micheau, 1989). Specific cellular component alteration associated with aging, have been observed in the morphology of mitochondria and Golgi substance in the cells of submandibular and parotid glands of wistar rats ranging in age from 100 to 997 days (Kurtz, 1954). Scott (1986) reported degenerative structural changes in the secretory tissues of most salivary glands in man with advanced aging. Mankapure, (2007) reported age related changes in ultra structure of salivary glands.

B. Biochemical changes:

Along with digestion and maintenance of oral health, the salivary glands also play a vital role in development and maintenance of various organs in the body (Barka, 1980) by producing number of biologically active polypeptides (Bodner and Baum, 1984). Salivary glands secrete various proteins, enzymes, glycoproteins, growth factors and pharmacological substances. The literature on the aging of salivary glands revealed that normal salivary glands functions are altered with aging (Storer, 1978) with decreased salivary flow rates and diminished saliva (Ship et al, 1995; Bretz et al, 2000 and Sonesson et al, 2003) accompanied with dry mouth. Aging reduces salivary secretion which results into oral infection and dental carries (Ship et al, 2000; Gezzi and Ship, 2003). The age related decrease in the salivary flow rates have been observed in elderly female, current smokers and those having complaints of dry mouth (Bretz et al, 2000; Bourdiol et al, 2004). Dayan et al, (2002) observed age related changes in the proliferative markers and various authors observed decrease in the flow rates as well as protein content of the secretions (Wu et al, 1994; Przybylom et al, 2004).
Significant age related decrease in stimulated and unstimulated submandibular and sublingual glands, saliva was observed in total population (Navazesh et al, 1992; Percival et al, 1994; Yeh et al, 1998; Hiratsuka et al, 2002 and Seikaly et al, 2004).

One of the consistent change that occurred in many salivary glands of aged animal is decline in the rate of synthesis of proteins and m-RNA. Bogart, (1970) showed diminution in RNA content and increase in acid phosphatase activity. Pillai et al, (2001) reported decrease in alkaline phosphatase activity in sialoadenectomised and sialoadenectomised-diabetic mice.

Study in age related changes in the cellular content of secretory proteins and protein synthesis in parotid salivary was decline with age (Kim et al, 1980; Kim, 1981). Koller et al, (2000) studied desipramine induced decrease in the glandular functions accented with age such as changed DNA and RNA synthesis and decrease EGF levels in all the age groups. Brian et al, (1981) found significant age related decrease in the submandibular gland contents of proteins, sialic acid and neutral sugars. Kobayashi et al, (2004) observed decrease in IGF-1 protein level and IGF-1-m-RNA level with age in senescence accelerated mouse submandibular gland. Significant age associated decrease in histatin concentration and secretion in submandibular and sublingual glands was observed by Johnson et al, (2000).

Glycoprotein content of salivary glands were studied in relation to age by Scott & Dorling, (1965) in the sublingual and Firat et al, (2006) in the submandibular glands of healthy normal subjects and found sex related differences in their glycoproteins. Pillai et al, (1998) while detecting the role of testosterone in synthesis of glycoproteins in acinar cells of salivary glands reported age related changes in salivary glands glycoproteins and found that concentration of hexoses and sialic acids

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were low in juveniles and increased in juveniles adults and were maximum in adults. Sonavane, (2007); and Mankapur, (2007) reported decrease in glycoprotein content of submandibular and sublingual glands of D-galactose stressed and naturally aged male mice. Tomake, (1998) reported decrease in glycoprotein content of salivary glands with age.

The enzyme content of saliva is reduced with age. Reduction in the content of amylase in mixed saliva has been reported in elderly persons (Mayer and Necheles, 1940; Chilla et al, 1974). Baum et al, (1982 a, b) showed alterations in an exocrine secretory proteins like α-amylase, the major secretory proteins of rat parotid gland about 50% reduction in amylase in old rats. Ben-Areyh et al, (1986) reported significant decrease in amylase activity in resting and stimulated parotid salivary glands of old human beings. Tomake and Pillai, (1996) studied amylase and trypsin of salivary glands in mice and found decreased amylase activity in old mice. Mote et al (2009) reported decreased amylase activity in D-galactose induced aging male mice. Histochemical data provided by Bogart, (1967) have demonstrated age related decrements occurring with various enzymes such as succinic dehydrogenase, non-specific esterase, cholinesterase and alkaline phosphatase in rat submandibular glands. Pillai et al, (2003) demonstrated decreased esterase activity in mice during aging. Mahay et al, (2004) showed significant age related decrease in amylase release by parotid acini of old mice.

All above structural and biochemical changes in salivary glands were mainly due to increased oxidative stress (Oded et al, 2007) during aging which in turn modify the immune system and harm the adaptability of the salivary glands and also physiology of other organs too.

The loss of immunity leading to dental carries. The common consequences of old age due to salivary dysfunction are saliva becomes thick and stringy, sores or split skin at the corners of mouth, bad breath,
difficulty in speaking and swallowing, a burning or tingling sensation on the tongue, altered sense of taste, increased plague, tooth decay, gum disease and other symptoms of Sjogren’s syndrome such as sialadenitis (increased bacterial, viral salivary gland infections). Mumps can infect parotid gland, causing xerostomia and sialothiasis (salivary stones), innocoeles (swelling of lower lip), and ranulus (mucocytes occurring in the submandibular and sublingual glands).

These salivary gland senescent changes also affect physiology of other organs like digestive system, endocrine system and reproductive system too. The main reason of salivary gland dysfunctions is free radicals damage which increased during aging due to imbalance between free radicals and their removal by cellular and antioxidant system.

8. Effect of Drugs on Salivary Glands:

Saliva is important for maintaining oral health and functions. There are evidences when medical therapy is intended to decrease saliva flow. The most common forms of therapy that interfere with salivation are drug therapies, radiation, chemotherapy and surgical therapy (Olver, 2006). Ionizing radiations can cause permanent damage to salivary glands that is manifest as acinar cell destruction with subsequent atrophy and fibrosis of the glands. Salivary dysfunction in older adults is likely to systemic diseases, prescription and non prescriptions medications, chemotherapy and head and neck radiations (Ship et al, 2002).

The drug therapies can affect salivation by a number of different mechanisms such as disruption of autonomic nerve function related to salivation, interference with acinar or ductal cell functions related to salivation cytotoxicity, indirect effects (Vaso constriction/Vaso dilation) fluid and electrolyte balance (Scully, 2003).

Drugs that decrease production of saliva include certain antidepressant, antihistamines, antipsychotic, sedatives, methyldopa and
diuretics (Streckfus, 1995). Persistent dry mouth is usually a symptom of a disorder of a side effect of drugs. Many drugs and drug classes have been linked to xerostomia. Xerogenic effect increases when many drugs are taken concurrently (Sreebny & Schwartz, 2008). Naglar and Hershkovich (2005) reported oral sensorial complaints in elderly individuals who use drugs. Drugs especially those with anticholinergic activity against the M3 muscarinic receptor are the most common cause of reduced salivation (Scully, 2003).

Koller et al., (2000) reported that desipramine decreased epidermal growth factor levels in all age groups but increased secretion of peroxidase and lysozymes. In desipramine treatment total RNA decreased with age in female NIA Fischer 344 rats. Significant reduction in case of enzymatic activities of phosphorylase total ATPase and Na⁺-K⁺-ATPase, cyclic AMP–specific phosphodiesterase and succinate dehydrogenase activities were suppressed by administration of isoproterenol in submandibular salivary gland of female rat (Rival et al., 2000). Pajukoski et al., (2001) reported complaints of dry mouth among the patients using psychiatric drugs. The amount of salivary taurine decreased by chronic treatment of muscarinic drugs like propantheline in mammalian cells affects the salivary functions (Mozaffari and Borke, 2002). Ghezzi & Ship, (2003) reported that salivary gland output was more adversely affected by an anti-sialogogue (glycopyrrolate) in healthy older than younger adults. There are some drugs which can improve salivary functions but are associated with adverse effects. Rasmussen, (1970) reported that isoproterenol (IPR) a β-adrenergic drug elicits salivary secretion. Baskerville et al., (1976) compared the effects of isoprenaline and pilocarpine on salivary glands of pigs and found that both drugs produced a significant increase in acinar area and weight of the parotid and submaxillary glands but no change in sublingual weight. Isoprenaline
did induce an increase in area of sublingual acini. It has been shown a single injection of isoproterenol in to rodent’s results in the marked stimulation of DNA synthesis in the salivary gland (Barka, 1980). Denny et al, (1991) reported increased mucin levels in submandibular glands of aged male mice after chronic isoproterenol treatment. Mozaffari and Borke, (2002) reported increased salivary secretion with pilocarpine treatment. Pillai and Walvekar, (2005) reported significant increase in protein, lipid, DNA content and LDH activity in isoproterenol administrated mice.

Drug amifostine used before radiation therapy is a thiol free radical scavenger has been shown to reduce the damage to salivary glands but it also induce nausea and hypotension (Sasse et al, 2006). Gornitsky et al, (2004) reported that pilocarpine do not improves salivary flow.

Pilocarpine hydrochloride is a para-sympathomimetic drug used to treat radiotherapy induced salivary gland damage. It can increase salivary flow after radiotherapy but is associated with adverse side effects such as headache, sweating, passing urine frequently (Greenspan & Daniels, 1987).

From above review of literature on effects of drugs on salivary glands, it was noticed that all these synthetic drugs have adverse side effects on salivary gland functions earlier or later affect other body organs and systems. So it was essential to find the drugs which can protect the salivary gland from oxidative stress during aging.

9. Choice of Plant:

The available research data suggest that W. somnifera (L.) Dunal, commonly called Ashwagandha (Sanskrit) is an Ayurvedic Indian medicinal plant. It is one of the most commonly used herb not only as antistress and adaptogenic agent but is also known to increase life span and delay aging. Of all the medicinal plants used in India’s Ayurveda, W.
*somnifera* is most highly prized. It has been widely used as home remedy for several ailments (Owais *et al.*, 2005; Sharma *et al.*, 1986) such as debility (Gupta *et al.*, 2003), tuberculosis (Kirtikar & Basu, 1935; Nadakarni, 1954), rheumatism (Chopra *et al.*, 1965) Parkinson’s disease, diabetes (Rajashankar *et al.*, 2009), cancer (Stan *et al.*, 2008), asthma (Etheretia *et al.*, 2009).

Screening and development of drugs for their antioxidant and antiaging activity is still in progress and there is much hope of finding antiaging and antioxidant drugs from indigenous medicinal plants. There is a need to search appropriate protective agent against aging and oxidative stress without any side effects. This search can be focused on plants used in traditional medicine and/or natural products that may offer treatment for aging than currently used synthetic drugs.

The chemical composition, pharmacological and therapeutic efficacy of *W. somnifera* has been well established (Dhuley, 2000). The roots and leaves of *W. somnifera* contain several alkaloids, withanolides, a few flavonoids and reducing sugars (Ganzer *et al.*, 2003) and are also rich in iron (Mishra *et al.*, 2000). The major bioactive chemical principle of *W. somnifera* is glycowithanolides including, Sitoindoside VII–X and withaferin (Bhattacharya *et al.*, 1997). This steroidal molecule are believed to account for the multiple applications of Ashwagandha (Bhattachayara *et al.*, 1997; Naidu et al., 2006), anti-inflammatory properties (Etheresia *et al.*, 2009) and has beneficial effects in treatment of arthritis, geriatric problems (Asthana & Raina, 1989), stress (Grandhi *et al.*, 1994). So glycowithanolides are of great interest to researchers. Toxicity studies on Ashwagandha reveal that it appears to be safe compound. Various constituents of Ashwagandha exhibit a variety of therapeutic effects with no any associated toxicity (Simon, 2000).
However, despite of these observations of diverse medicinal activities attributed to this plant, very less research are carried on secretory organs. Salivary glands are major secretory glands which affect the most after brain during aging.

The present study was undertaken to investigate the effect of glycowithanolides from *W. somnifera* leaves extract on salivary glands of D-galactose induced male mice by studying various biochemical, histological and histochemical parameters.

10. **Choice of Drugs:**

A. **D-galactose:**

D-galactose is used as an aging inducing agent, it is also called as brain sugar whose levels along with L-galactose (both are AGEs) are found to be elevated in aging cells. Their increasing concentrations in turns accelerate the natural aging process by increasing Reactive Oxygen Species (ROS) accumulation in the neuronal cells. D-galactose induced impairment of neurogenesis which is similar to natural aging process (Song *et al.*, 1999). It also induces quick aging in brain and heart of young mice (Deshmukh *et al.*, 2006). In their study, Kalmade *et al.*, (2007) in prostate gland; Sonavane, (2007) in salivary glands; Pillai *et al.*, (2008) in brain showed induced stress in these organs. Mote *et al.*, (2009) also showed decreased amylase and proteins in the salivary glands in D-galactose stressed mice. Increase in lipid peroxidation (Deshmukh *et al.*, 2006; Vora *et al.*, 2009) in brain and heart of D-galactose stressed mice and decrease in antioxidant enzymes in cytoplasm (Vora, 2005). Therefore D-galactose was selected to induce aging in mice.

B. **Centrophenoxine:**

It is antiaging drug which is a brain-metabolic stimulant (neurotrophic drug). Its dimethylamino-ethanol moiety is an efficient OH⁻ radical scavenger (Zs-Nagy & Nagy, 1980; Zs-Nagy & Floyd, 1984)
and provides free radical protection in the old nerve cells. It increases total proteins and m-RNA synthesis rate in the brain cortex. Centrophenoxine treatment is able to prolong the medium life span and to improve the learning ability (Zs- Nagy, 1989). Age related changes can be improved by centrophenoxine. So it was used for comparison with antiaging property of glycowithanolides—a natural antioxidant from *W. somnifera*.

C. Glycowithanolides:

It is the natural antioxidant, bioactive chemical principle of *W. somnifera* (Bhattacharya *et al*, 1997; Ghosal *et al*, 1989) consisting of sitoindoside VII–X and withaferin. Glycowithanolides reported to have antistress properties (Grandhi *et al*, 1994), antioxidant properties (Bhattacharya *et al*, 2000) and therapeutic and antiinflammatory properties (Etheresia *et al*, 2009). These steroidal molecules are believed to account for the multiple clinical applications of Ashwagandha. So it was used to study the protection of salivary glands during aging.

11. Choice of Animal:

For the present study male albino mice, *Mus-musculus* were used because these animals have been used for studies in pharmacology, toxicology and psychology since long ago. Their physiology resembles to that of human physiology and their genetic setup also shows similarities with human being. As the study is on aging, the animals with short life span are selected. *Mus musculus* has the life span of two and half year ± six months is being used to study effect of antioxidant. Male mice were selected because female’s estrogen provides natural protection against free radical damage but in case of male there is no estrogen, so male mice was used to study the effect of free radical damage during induced aging and natural aging stress.
12. Choice of Organs:

Aging affects the growth, differentiation, structure and functions of various organs in the body. Though the important organs like brain and heart are considered to be the major sites of senescent changes, salivary glands are also adversely affected during aging. Salivary glands are not only the organs of digestion and oral health but they synthesize and secrete many enzymes, growth factors and glycoproteins which are essential for normal development and maintenance of post mitotic cells in the body. These products are decreased during aging which in turn affect other body functions (Bodner & Baum, 1984). Hormones like melatonin, testosterone, estrogen and thyroxin have a correlation with salivary functions, whose concentration decreases during aging (Ashour, 1998).

An important problem in modern medicine and biology is the study of mechanism of aging and possibility of development of diseases connected with them. There are many evidences of age changes in structural and histochemical parameters of salivary gland function in man and different species of animals (Rybakava, 1982; Mankapure, 2007; Sonavane, 2007). The salivary glands of mouse and rat serve as an important model to study aging, since it can be studied both in vivo and in vitro (Masanori & Edward, 1996). Salivary glands are readily accessible and well characterized; therefore they are useful tool for the study of normal aging process and impact of stress on organ reserve and secretary functions (Baum et al, 1992) and reflect many systemic conditions (Atkinson & Wu, 1994). The salivary gland is an expanding organ having most reserve capacity. We can induce the aging changes easily without obtaining its mortality quickly by inducing oxidative stress in it (Ghezzi & Ship, 2003). Physiology of salivary glands depicts physiological status of animals and human beings by increasing or decreasing their secretions. Therefore the metabolic organs like salivary glands were selected as the
target organ for the study of age changes due to D-galactose and natural aging and to study the arrest of these changes or reversal (if any) of these changes by giving potential phytoantioxidant like glycowithanolides from *W. somnifera*.

13. **Aim and Objectives of Proposed Work:**

After studying the importance of salivary secretion in animal physiology, effect of aging on salivary dysfunctions like

a. Xerostomia.

b. Decrease in various enzymes and growth factors required for maintenance of various body functions, growth and differentiation (in old age also, stem cells divide grow and differentiate).

The aim of present work was to decline aging process in salivary glands using phytochemical like ‘Glycowithanolides’ extracted from Ashwagandha, known as Indian ginseng.

To study protection of salivary glands during aging following objectives were designed and worked out

a. Study of morphology and structure of salivary glands.

b. Study of biochemistry of salivary glands

i. Enzymes

ii. Glycoproteins