Over the past several decades, there has been a tremendous amount of research directed at discovering the links between oral health and overall body health. Mouth is an easily accessible window to the body. The health status of our mouths can give us a strong indication of the health of our bodies. All teeth and gum infection have a bacterial origin. The bacteria that is associated with gum disease, especially in more advanced stages of it, is very toxic. This is very dangerous because the bacteria and toxins can enter the bloodstream even when the food is chewed, in the presence of gum infection. This bacterial infection can cause changes in body chemistry that may create clotting—which can lead to stroke.

3.1 Oral bacteria causative of other infections

Most studies have been done on skin, respiratory and urinary system pathogens and there has been little research about oral pathogens (Ahmad and Beg, 2001). Cases of bacteremia due to Capnocytophaga, Fusobacterium, and Leptotrichia species have been described in patients with cancer and in recipients of hematopoietic cell transplants, particularly in the presence of mucositis (Baquero et al., 1990; Fanourgiakis et al., 2003; Lark et al., 2001; Lin et al., 1998; Weinberger et al., 1991). In a study of bone marrow transplant recipients receiving quinolone prophylaxis, Fusobacterium nucleatum and Leptotrichia buccalis were the most common causes of anaerobic bloodstream infection (Lark et al., 2001). This case illustrates that anaerobic bacteria may produce atypical oral pathology, as well as bacteremia, in immunocompromised patients (Fredricks et al., 2005).

The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are byproducts of carbohydrate metabolism by Streptococcus mutans, a cariogenic bacterium (Loesche, 1986). Colonization of teeth by
cariogenic bacteria is one of the most important risk factors in the development of dental
diseases (Loesche, 1986). *S. mutans* and *Candida albicans* are the two microbes often
implicated in oral diseases, *C. albicans* is the most common yeast isolated from the oral
cavity and a common cause of oral thrush, endocarditis, septicemia, vaginitis and infection
of skin, nails and lungs (Agbelusi *et al.*, 2007; Bagg, 1999; Lee *et al.*, 2004). It is by far the
fungal species most commonly isolated from infected root canals, showing resistance to
intercanal medication (Odds, 1988; Oztan *et al.*, 2006). *Staphylococcus aureus* is a major
human pathogen, responsible for a number of hospital-acquired infections, initially
colonizes several locations in the human body, but the mouth and hands are the main
reservoirs for propagation of this pathogen in the hospital environment (Knighton, 1960;
Lowy, 1998; Piochi and Zelante, 1975). Individuals heavily colonized by cariogenic
bacteria are considered to be at high risk for dental caries. Hence eradication of these
microorganisms is important for dental treatment (Rodis, 2006).

The bacterial communities present in the oral cavity and the lungs of 19 adult cystic
fibrosis (CF) patients were compared by using terminal restriction fragment length
polymorphism analysis of 16S rRNA gene PCR products amplified from nucleic acids
extracted directly from bacteria in clinical samples. Sputum samples were not found to be
subject to profound contamination by oral cavity bacteria. These findings are consistent
with previous studies suggesting that the teeth can serve as a reservoir for respiratory
infection (Mojon 2002; no back ref Scannapieco and Genco, 1999 and Scannapieco *et al*.,
1998). Oral bacteria colonize the respiratory system of cystic fibrosis patients (Rogers *et al*.,
2006).

Traditionally, the detection of bacterial species within the cystic fibrosis (CF) lung
has relied on identification through the in vitro cultivation of the microbes within
expectorated sputum (Rogers *et al*., 2006). A number of species normally associated with
the oropharyngeal environment were identified in cystic fibrosis sputa (Rogers et al., 2003; 2004; 2006).

The relationship between dental plaque, oral colonization, and nosocomial infections was studied in 57 ICU patients. For each patient, quantitative cultures of dental plaque and tracheal aspirates, assessment of dental plaque on premolars, and assessment of nosocomial infection were done at the time of admission (day 0) and every fifth day until the patient died or was discharged from the ICU. (Fourrier et al., 1998) found high concordance between the bacteria present in cultures of dental plaque and the bacteria present in cultures of tracheal aspirates. A nosocomial infection developed in 21 patients. Five of these infections were pneumonias (determined by recovery of pathogens from bronchoalveolar lavage fluid). In 4 of 5 patients with pneumonia, the causative organism (A baumannii in 2 patients and P aeruginosa in 2) was isolated from the patients’ dental plaque before the diagnosis of pneumonia. Dental plaque can serve as a reservoir for microorganisms that can cause Ventilator associated pneumonias (Fourrier et al., 1998).

Oropharyngeal colonization is associated with several systemic diseases (Li et al., 2000; Gendron et al., 2000; Lockhart and Durack, 1999; Loesche, 1997), including cardiovascular disease (Fowler et al., 2001; Scannapieco, 1998), chronic obstructive pulmonary disease (Scannapieco et al., 1998), endocarditis (Seymour and Whitworth, 2002; Carmona, 2002; Hoen, 2002; Kitten et al., 2000; Burnette-curley et al., 1995; Munro and Macrina, 1993), and bacteremia (Marron et al., 2000; Kerr, 2000; Nieves et al., 1997, Lockhart, 1996).

The impact of oral conditions for halitosis patients and treatments in Italian subjects were studied. A self reported questionnaire was used to detect the self reported halitosis and other variables possibly linked to it, and a dental anxiety scale divided into two subscales that explore a patients dental anxiety and dental anxiety concerning dentist-
patient relations. The rate of self reported halitosis was 19.39%. Halitosis require professional care not only by dentists, but also psychological support as it is a problem that leads to avoidance behaviors and there by limits relationships. It was also linked to poor self care (Settineri, 2010).

A prospective study was conducted in the department of medical microbiology, Netherland to analyze the inadequate salivary flow and poor oral mucosal status in intubated intensive care units patients. In the study, 24 ventilated intensive care unit patients and 20 Coronary artery bypass graft patients were included. The dental hygienist examined the presence of periodontal disease and mucositis at admission and subsequently every week during their stay in ICU. At the same time, stimulated salivary flow and salivary total immunoglobulin. Oropharyngeal culture obtained Coronary artery bypass graft patients were examine the day before the operation, 1 day, 1 week and 2 weeks after surgery. The result showed,

- Temporarily reduced post operative stimulated salivary flow and total salivary immunoglobulin A output in coronary artery bypass graft patients and nearly absent stimulated salivary flow in intensive care unit patients.
- Oropharyngeal colonization with potentially pathogenic micro organisms in intensive care unit and not in coronary artery bypass graft patients.
- Increase in mucositis index and oropharyngeal colonization in intensive care unit.
- Absence and adequate salivary flow in intensive care unit patients cause severe xerostomia which contribute to development of mucositis and oropharyngeal colonization with gram negative bacteria (Dennesen, 2003).

3.2 Mechanism behind plaque and dental caries formation

Dental caries can be prevented by the effective antimicrobial agents against oral pathogens. The enzyme glucosyl transferase which is present in oral flora polymerizes
glucose (obtained from sucrose) to water insoluble glucans. The sticky polysaccharides enable the oral bacteria to adhere to the tooth surface. This leads to formation of plaque and causing dental caries. Dental caries can be inhibited by the inhibition of glucosyl transferase activity by specific enzyme inhibitor (Yanagida et al., 2000), inhibition of initial cell adhesion of *S. mutans* by polyclonal and monoclonal antibodies (Raamsdonk et al., 1995) and inhibition of cell growth of *S. mutans* by antibacterial agents have been investigated. Antibiotics such as penicillin and erythromycin have been reported to effectively prevent dental caries in humans (Kubo et al., 1992) but they are never used clinically because of many adverse effects such as hypersensitivity reaction, supra infections and teeth staining. The viridian group streptococci including *S. mitis, S. sanguis* and *S. mutans*, the most representative human carcinogenic bacteria are moderately resistant to antibiotics (Venditti et al., 1989).

### 3.3 Effects of Chlorhexidine against oral flora

Oral bacterial microcosms, established using saliva inocula from three individuals, were maintained under a feast-famine regime within constant-depth film fermenters. Steady-state communities were exposed four times daily, postfeeding, to a chlorhexidine (CHX) gluconate-containing mouthwash (CHXM) diluted to 0.06% (wt/vol) antimicrobial content. The microcosms were characterized by heterotrophic plate counts and PCR denaturing gradient gel electrophoresis (DGGE). CHXM caused significant decreases in total anaerobe and total aerobe/facultative anaerobe counts (*P* < 0.05), together with lesser decreases in gram-negative anaerobes. In conclusion, population changes in plaque microcosms following repeated exposure to CHXM represented an inhibition of the most susceptible flora with a clonal expansion of less susceptible species (McBain et al., 2003).

Colonization of the oropharynx by the pathogen that caused Ventilator Associated Pneumonia (VAP) was identified before the pneumonia was diagnosed. The effect of oral
health on the development of VAP in 66 patients in a medical ICU was studied. The number of organisms present in oral cultures increased from day 1 to day 4 and remained high on day 7. The patients with mechanical ventilation who had a VAP-related pathogenic organism in cultures of tracheal aspirates (including *S. aureus*, *S. pneumoniae*, *A. baumannii*, and *P. aeruginosa*) also had the same species in oral cultures before or concurrent with appearance of the species in the tracheal aspirates. Research findings indicate that the oral cavity is a primary source of pathogens that cause VAP (Munro and Grap, 2004).

Oral decontamination with chlorhexidine reduces the incidence of ventilator-associated pneumonia. Trial medication chlorhexidine, chlorhexidine / colistin and placebo (PLAC) was applied every 6 hours into the buccal cavity. Oropharyngeal swabs were obtained daily and quantitatively analyzed for gram-positive and gram negative microorganisms. Endotracheal colonization was monitored twice weekly. The daily risk of Ventilator associated pneumonia was reduced in both treatment groups compared with placebo group 65% confidence interval for chlorhexidine and 55% for chlorhexidine / colistin. Chlorhexidine / colistin provided significant reduction in oropharyngeal colonization with both gram negative and gram positive microorganisms. Topical oral decontamination with chlorhexidine or Chlorhexidine / colistin reduces the incidence of Ventilator associated pneumonia (Koeman et al., 2006).

An early study demonstrated that oral treatment of human volunteers with CHX resulted in a 30 to 50% reduction in total bacterial counts with an associated reduction in counts of *Streptococcus mutans* (Schio¨tt et al., 1976) no back ref. Recent reports have demonstrated that the chlorinated diphenylether antibacterial triclosan (TCS) can select for mutants in the FabI gene of *Escherichia coli* at sublethal concentrations (Levy et al., 1999; Levy, 2000; McMurry et al., 1998) that confer overt TCS resistance. Although the evidence is still ambiguous, assertions have been made that other antimicrobials might similarly
select for resistance and that biocides in general could affect susceptibilities to chemically unrelated compounds (Fraise, 2002; Levy, 2000; Loe and Schiott, 1970; Schweizer, 2001).

A randomized, double blinded placebo controlled clinical trial study on efficacy of a low-concentration chlorhexidine mouth rinse in non-compliant periodontitis patients was conducted. Forty-seven patients (22 placebo and 25 test group) participated. After 3 months, plaque levels increased in the placebo group, while diminished in the test group (p<0.001). Similar effects were found for bleeding on probing. The other clinical parameter did not show significant differences. Microbiological variables demonstrated inter-group significant reductions in subgingival counts of fusobacterium nucleatum and prevotella intermedia and a decrease of the total bacterial counts in saliva (Escribano et al., 2010).

The antimicrobial properties of ten commonly available mouthwashes were studied against four oral pathogens. Hexidine mouthwash emerged as the most effective mouthwash [maximum mean diameter of inhibition zone against S. aureus (28.3mm to 33.9mm) followed by S. mutans (23.6mm to 26mm), S. cerevisiae (20.6mm to 26.3mm) and minimum against C. albicans (11.9mm to 22.9mm)] followed by Chlohex and Triguard, all of which had excellent level of activity. Zytee, Chlohexplus, Hexnor and Chlorhexidine that showed good antimicrobial activity and finally, displaying very little antimicrobial activity was Listerine while Toss-K and Senquel-AD totally lacked antimicrobial activity. It is concluded that hexidine mouthwash (ICPA Health Products Ltd., Ankleshwar, India) showed excellent antimicrobial activity against the four dental caries causing microorganisms in vitro (Kamal Rai et al., 2010).

The effectiveness of povidone Iodine mouth care on oral hygiene among intubated patients was assessed at selected hospital, Kerala. The study was conducted over one month 60 patients were participated among 30 patients assigned to experimental group and 30 patients were assigned to control group. Oral care given with povidone iodine solution in
experimental group for four days, whereas in control group patients didn’t had standard oral care as per hospital policy. The “t” value was 6.55, which is significant at $P<0.05$ level. The result showed there was effectiveness of povidone iodine mouthcare on oral hygiene among intubated patients than control group (Sowmya Mohan Das, 2010).

3.4 Risk of alcohol in mouthwashes

Chronic excessive alcohol consumption is a strong risk factor for cancer of the upper aero-digestive tract (oral cavity, pharynx, hypopharynx, larynx, oesophagus), the liver, the colo-rectum and the breast. A great number of epidemiological studies have demonstrated the correlation between alcohol ingestion and the occurrence of cancer in these organs (Seitz et al., 1998). The exact mechanism of ethanol-associated carcinogenesis has remained obscure, since ethanol is not a carcinogen. There is increasing evidence that acetaldehyde (AL) rather than alcohol is responsible for the co-carcinogenic effect of alcohol (Seitz et al. 2001). In the gastrointestinal tract AL can be generated from ethanol through the action of mucosal and/or bacterial alcohol dehydrogenase (Seitz & Oneta, 1998). AL is highly toxic, mutagenic and carcinogenic. AL interferes with DNA synthesis and repair at many sites and can consequently result in tumour development (Anonymous, 1985).

3.5 Alcohol free mouth rinses

Alcohol-containing mouthwash has been clinically shown to be an effective antibacterial agent, but many people are unable to use this product (Radford et al., 1997) because, the amounts of alcohol contained in mouthwashes and the burning sensation they cause (Sharma et al., 2003) to children, diabetics, alcoholics, and members of certain religious faiths (Radford et al., 1997) such persons are recommended to use alcohol-free brands, such as ones that contain CPC. Commercial mouthwash brands typically use alcohol as a main ingredient to improve the antibacterial
effects of the mouthwashes. Many alcohol-free mouthwashes use cetylpyridinium chloride (CPC) as an active antibacterial ingredient. It is effective because it is a cationic surface active agent that is able to absorb the negatively charged phosphates of the bacteria’s cell membrane (Radford et al., 1997).

The antibacterial effect on the oral flora was studied using cetylpyridinium chloride (CPC). The researchers compared the antibacterial effectiveness of a 0.05% CPC solution and a placebo. The 132 participants rinsed with 10 ml of a given mouthwash (either CPC or placebo) for 30 seconds twice daily; once in the morning and once in the evening. After the six week study, the researchers found that the CPC solution did not reduce or increase the amounts of S. mutans in the oral cavities of the participants (Radford et al., 1997).

The antibacterial effect of cetylpyridinium chloride (CPC) was studied using higher concentrations. In this study, 0.07% concentration of CPC was used. The study was conducted on several different oral bacteria including S. mutans. The CPC solution exhibited 99% bacteria kill for all bacteria tested. An in vivo study was conducted with same concentration of CPC mouthwash against a positive control Listerine and a negative control. Participants rinsed with 20 ml of an assigned mouthwash for 30 seconds in the morning and in the evening. 25 % plaque reduction was noticed when compared to the placebo in just 4 days. These results show the effectiveness of a CPC solution at a 0.07% concentration (Witt et al., 2005).

The parameters which are of significant importance in regulating homeostasis in the mouth include mainly the integrity of the host defenses (including saliva flow) and the composition of the diet. Consumption of food with high fermentable sugar content have greater proportions of mutans streptococci and lactobacilli in plaque. Antimicrobial peptides are though recognized as important components in controlling microbial populations in the mouth, much is not known about their role in regulating the resident
microflora at sites in the body, but risk of caries is known to increase with reduction in their activities. It has been reported that their role is complex because they are multifunctional and have more than a mere antimicrobial action, e.g., by linking the innate and adaptive arms of the immune response (Devine, 2003).

3.6 Routine Oral care

The impact of routine oral care on opportunistic pathogens in the institutionalized elderly was studied. Twenty five elderly subjects participated in the study. Dental hygienists cleaned the mouth of the subjects by routine and professional oral care techniques and opportunistic pathogens were collected in oral cavity by using cotton swab. The species of microbes were determined. The result revealed that professional oral care was effective for reducing infections. This shows that the importance of regular oral care in cleaning hard and soft surfaces of the oral cavity improves oral health in institutionalized elderly people (Kokubu, 2008).

3.7 Herbal extracts against oral pathogens

The more potent herbal extract which would be effective against *Streptococcus mutans* was studied and it was compared with chlorhexidine. The herbal extracts include thyme, mint, garlic, cinnamon, chamomile, tea tree, clove, and spearmint, sage and rosemary. *Streptococcus mutans* was cultured in blood agar medium. Chlorhexidine was used as positive control, while methanol and blank discs were used as negative controls. Finally rosemary was found to be a potent antimicrobial plant against *Streptococcus mutans*. The authors suggested for more studies with plants for production of herbal mouthwashes (Dalirsani et al., 2011).

The extracts of six medicinal plants namely *Azadirachta indica*, *Emblica officinalis*, *Terminalia belerica*, *Terminalia chebula*, *Terminalia arjuna* and *Mangifera indica* L, were used as individual and as combination to test the Total Viable Counts of
dental plaque and the animal toxicity, anti-microbial activity were also analyzed. The formulation of mouthwash was then prepared and its anti-microbial activity was compared with the marketed chlorhexidine mouthwash. The results indicate that this poly-herbal mouthwash improves the oral hygiene in healthy individuals and help in preventing dental caries and gingivitis through plaque control (Atul et al., 2011).

Antibacterial activity of the extract of *Moringa oleifera* was evaluated against various selective oral bacteria using Well diffusion technique. The extract was made into 100mg/ml solution and then filtered. The extract inhibited the *Streptococcus mutans* (MTCC 890), *Streptococcus mutans* (MTCC 497), *Streptococcus salivarius* (MTCC 1938), *Streptococcus mitis* (MTCC 2696), *Lactobacillus fermentum* (MTCC 903), *Streptococcus anginosus* (MTCC 1929), *Streptococcus gordonii* (MTCC 2695), *Lactobacillus acidophilus* (MTCC 447) and *Staphylococcus aureus* (MTCC 96). The most significant effect was seen against *S.aureus*, which are important oral pathogens. Purification of methanol extract of the leaf of *M.oleifera* afforded ß sitostiro, Niazinin A, Stigmasterol, Kaempferol-3- o-ß-D-Glucopyranoside and Quercetin-3-o-ß-D-Glucopyranoside. All the isolated compounds from *M.oleifera* were active against among *Streptococcus mutans* (MTCC 497), *Streptococcus salivarius*, *Lactobacillus fermentum*, *Streptococcus anginosus*, *Streptococcus gordonii*, *Lactobacillus acidophilus* (Koteswara Rao et al., 2011).

The susceptibility of the aqueous extract of *Piper betle* towards seven species of oral Candida was screened out. It was found that *P. betle* extract exhibited high antifungal activities towards *Candida albicans, Candida tropicalis, Candida glabrata, Candida dubliniensis, Candida lusitaniae, Candida krusei* and *Candida parapsilosis*. The results obtained have shown the potential use of *P. betle* extract as antifungal agent and thus significantly contribute to its antifungal development (Himratul- Aznita et al., 2011). Thus, the plant extract can be added as an ingredient in oral related products.
The antibacterial effectiveness of 10 mouthwashes was studied against Streptococcus mutans, a common oral bacterium. The mouthwashes tested were Listerine, Plax, Walgreen’s Fresh Breath, Walgreen’s Fluoride, Viadent, Dr. Tichenor’s, Scope, Targon, Breath Rx, and Crest Prohealth. Four active ingredients were also tested at three different concentrations per active ingredient, cetylpiridinium chloride (0.05%, 0.07%, 0.10%), alcohol (10%, 20%, 30%), menthol (0.02%, 0.05%, 0.10%), and thymol (0.05%, 0.07%, 0.10%). The average zone of inhibition was measured in millimeters from the mouthwashes and active ingredient concentrations; the data were collected from 20 replicates per treatment after an incubation period of 24 hours at 37°C. The result supported the findings that the cetylpyridinium chloride-containing mouthwashes had higher antibacterial effectiveness (Saint Martin’s University, 2007).

3.8 Botanical description of Glycyrrhiza glabra

Glycyrrhiza glabra is a tall, erect perennial herb, with a sweet tasting ingredient Glycyrrhizin, which is 50 times sweeter than sugar. It belongs to Fabaceae/ Leguminoseae family. The strong woody root stock is about 1 cm thick, brown on the external surface and yellow on the internal surface. The sweet taste is present in the roots (Fletcher, 1991 no back ref; Mills, 1991; Weiss and Fintelmann, 2000) no back ref. The major part of rhizome which is used for therapeutic value is the dried roots and stolon (Hoffmann, 2000; McIntyre, 1994, Mills, 1991). The branches of the plant bears 3-7 pairs of opposite, short stalked, pinnate leaves with a single leaf at the tip. Clusters of bluish – purple butterfly shaped flowers with white tip was seen from the axils of the leaves (Fletcher, 1991 no back ref; Mills, 1991; Weiss and Fintelmann, 2000) no back ref. The fruits are small smooth skinned leguminous seedpods, which are 2- 2.5cm long. Each contain three or four seeds (Fletcher, 1991 no back ref; Mills, 1991).
3.9 Bioactivity of some phytochemical constituents of *Glycyrrhiza glabra*

Many constituents with antioxidant activity was isolated and purified from *Glycyrrhiza glabra*. The isolated compounds were identified as the isoflavans, Hispaglabridin A, Hispaglabridin B, Glabridin; the two chalcones, isoprenyl chalcone derivative isoliquiritigenin; theisoflavone, formononetin. Among these compounds, Glabridin constituted the major component in the crude extract and the most potent antioxidant toward LDL oxidation (Fuhrman *et al.*, 1997).

Glycyrrhiza glabra could attenuate peroxynitrite induced renal oxidative damage through inhibition of protein nitration (Yokozawa *et al.*, 2005). Antioxidant capacity of licorice is used to treat kidney or urinary system based on oxygen radical absorbance capacity method (Wajcikowski *et al.*, 2007) Possible role of Nrf2 (Nuclear erythroid 2p45-related factor), a key transcription factor of phase II drug metabolizing enzymes in renal cellular defense against oxidative stress has been suggested in mice (Kanki *et al.*, 2008). It was concluded that, *G. glabra* could reduce acetaminophen-induced hepatorenal damage in mice under lab conditions probably by strengthening endogenous antioxidant defense in the liver and kidneys of mice (Anjali Sharma and Rathore, 2011).

Glabridin is a natural polyphenolic isoflavone of Licorice root. It has antioxidant activity, i.e., it can scavenge the free radicals that could promote a decrease in lipid peroxidation and could protect LDL from oxidation (Carmeli *et al.*, 2008). This occurs through a direct interaction with the lipoprotein and/or an indirect effect through accumulation in arterial macrophages. Therefore licorice represents a potent nutrient, which can attenuate the development of atherosclerosis, secondary to its antioxidant properties against lipids peroxidation in arterial cells (Vaya *et al.*, 1997).

The isolates from GG roots viz. glabridin (an isoflavan) and isoliquiritigenin (a flavonoid), are known to be pharmacologically active compounds. Glabridin is reported to
be a potent antioxidant towards LDL oxidation, whereas isoliquiritigenin is known to exert vasore-laxant effect, anti-platelet, anti-viral, estrogenic activities and has the protective potential against cerebral ischemic injury. Antihyperlipilaemic and antihypertriglyceridaemic properties of *Glycyrrhiza glabra* root have also been reported. *Glycyrrhiza* flavonoids provide protection to hepatocytes exposed to carbon tetrachloride, and galactosamine (Geetha and Lakshmi, 2011). Glycyrrhizin may reduce the growth and acid production of oral bacteria, but results have varied. Other experiments suggest that inhibition of bacterial adherence and inhibition of the enzyme required for plaque formation may be alternative mechanisms for the anticariogenic action of licorice (Isbrucker and Burdock, 2006).

Constituent properties of licorices derived from *Glycyrrhiza uralensis*, *G. glabra*, and *G. inflata* are revealed by comparing 117 of licorice identified using four genetic markers; internal transcribed spacer (ITS) on nuclear ribosomal DNA, *rbcL* gene, *matK* gene, and *trnH–trnK1* intergenic region on chloroplast DNA. Regarding six main constituents of licorice; glycyrrhizin, liquiritin, liquiritin apioside, isoliquiritin, isoliquiritin apioside, and liquiritigenin, the constituent property of *G. glabra* resembles to that of *G. inflata*. On the other hand, the constituent property of *G. uralensis* is not similar to that of *G. glabra* or *G. inflata* and is characterized by a wide content variation of the six constituents compared to those of *G. glabra* and/or *G. inflata*. When licorice is used for medicinal purposes, the licorice species should be selected with recognition of those constituent properties. In the assessment of the six kinds of main constituent contents, *G. glabra* and *G. inflata* can be used equally as medicine, but *G. uralensis* might be not able to use similarly with *G. glabra* or *G. inflata* (Kenji et al., 2007).

In the course of screening for antifungal compounds from various plant material, licorice (*Glycyrrhiza glabra*) extracts with 80% methanol (oil-based extract of licorice;
OEL) was found to have high fungicidal effect against *Arthrinium sacchari* M001 and *Chaetomium funicola* M002, and its active compound was identified as glabridin (3-(2’,4’-dihydroxyphenyl)-8-dimethylpyrano (8,7-e]chroman) (Hiroshi and Jun, 2002). The compound Glabridin has been reported to exhibit multiple pharmacological activities such as cytotoxic activity, antimicrobial activity, estrogenic and anti-proliferative activity against breast cancer cells. It also affects melanogenesis, inflammation, low-density lipoprotein oxidation and protection of mitochondrial functions from oxidative stress (Choi et al., 2005). Under optimum extraction conditions, 0.92mg/g of glabridin can be extracted from licorice and the recovery was 72.5% (Tian et al., 2008).

The anti-fungal activity of *Glycyrrhiza glabra* was tested and it possesses good anti fungal activity. Antimycobacterial activity of *Glycyrrhiza glabra* was found at 500 mg/mL concentration in a study. Bioactivity guided phytochemical analysis identified glabridin as potentially active against both *Mycobacterium tuberculosis* H(37)Ra and H(37) Rv strains at 29.16 mg/mL concentration. It exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria. The results indicate potential use of licorice as antitubercular agent through systemic experiments and sophisticated anti-TB assay (Gupta et al., 2008).

The levels of various antioxidants in saliva to identify differences between the saliva of patients with healthy peri-implant tissues and patients with peri-implant disease were studied. The total antioxidant status of saliva and concentration of uric acid and ascorbate, which are the main salivary antioxidants, were significantly decreased in patients with peri-implant disease. The authors suggest that the treatment of periimplant disease may involve adjuvant antioxidants supplementation together with the recommended treatment. Numerous antioxidants have been tried and tested both by systemic administration and as mouthwashes. These include synthetic products, like vitamins, and
natural products like wine and green tea. As described previously, naturally occurring substances in higher plants have antioxidant activity, which are of great application in the control of chronic disorders caused by oxygen containing free radicals (Liskmann et al., 2007).

3.10 Treatment of oral diseases with Glycyrrhizin

Oral lichen planus, an inflammatory disease is often experienced by patients with chronic hepatitis C. It leads to lymphocytic hyperkeratosis of the oral mucosa. It is rarely cured and effective treatments are limited. The patients with oral lichen planus were given either routine dental care or 40 mL IV glycyrrhizin daily for one month. 66.7% of patients noted improved clinical symptoms, such as decreased redness, fewer white papules, and less erosion of the mucosa. In the non-glycyrrhizin group only one 14.3% reported any improvement (Daganagao et al., 1996).

Dried, aqueous extract of licorice root, containing 108, 217, 380 and 814 mg of glycyrrhizin was administered to 4 groups of 6 healthy volunteers of both sexes for 4 weeks. No significant effects occurred in groups 1 and 2. After 2 weeks, side effects leading to withdrawal from the protocol occurred in a female in group 3 (headache), a male with a family history of hypertension in group 4 (arterial hypertension), and a female also taking oral contraceptives in group 4 (hypertension, hypokalaemia and peripheral edema). In group 4, transient reduction in kalaemia and increase in body weight were found after 1 and 2 weeks, respectively. A depression of plasma renin activity occurred in groups 3 and 4. In healthy subjects, only the highest doses of licorice led to untoward effects. These were favored by subclinical disease or oral contraceptives, and were less common and pronounced than what has been reported after the intake of glycyrrhizin taken as such or as a flavoring agent in confectionery products (Bernardi et al., 1994).
3.11 Pharmacological activities of *Glycyrrhiza glabra*

The effect of licorice on serum testosterone in nine healthy women aged 22-26 was studied using licorice preparation and found that the total serum testosterone decreased from 27.8 ([ or -] 8.2) to 19.0 ( or -] 9.4) ng/dL after one month, and further decreased to 17.5 ([ or -] 6.4) ng/dL after the second month of therapy. This is likely due to inhibition of 17-hydroxysteroid dehydrogenase, indicating licorice may be of benefit in treating women with hirsutism and polycystic ovary syndrome (Armanini, *et al.*, 2004).

Numerous viruses cause latent infections in humans, and reactivation often results in pain and suffering. While vaccines for several of these viruses are available or currently being studied in clinical trials, and antiviral therapies have been successful in preventing or treating active infection, therapy to eradicate latent infection has lagged behind. A new study reported in this issue of the JCI shows that treatment of cells latently infected with Kaposi sarcoma-associated herpes virus (KSHV) with glycyrrhizic acid, a component of licorice root, reduces synthesis of a viral latency protein and induces apoptosis of infected cells. This finding suggests a novel way to interrupt latency (Cohen, 2005).

The extract of the trunk of *Glycyrrhiza glabra* exhibited high tyrosinase inhibitory activity. Tyrosinase is responsible for melanin biosynthesis (Lerner and Fitzpatrick, 1950; Mason, 1948). It is the key enzyme involved in first stage of melanogenesis pathway catalyzing the conversion of L-tyrosine to L-dopaquinone (Marmol *et al.*, 1993).

After oral ingestion, glycyrrhizin is first hydrolyzed to 18b-glycyrrhetinic acid by intestinal bacteria, and after complete absorption from gut, 18beta-glycyrrhetinic acid is metabolized to 3beta-monoglucuronyl-18beta-glycyrrhetinic acid in the liver. This metabolite then circulates in the bloodstream. The main part is eliminated by bile and only a minor part (0.31–0.67%) by urine. After oral ingestion of 600 mg of glycyrrhizin the metabolite appeared in urine after 1.5 to 14 hours. Maximal concentrations (0.49 to 2.69
mg/l) were achieved after 1.5 to 39 hours and metabolite can be detected in the urine after 2 to 4 days (Glavac and Kreft, 2012) no back ref.

The anti-inflammatory compound Glycyrrhizin (GL) isolated from *Glycyrrhiza glabra*, has been identified as a thrombin inhibitor. The in vivo effects of GL upon induced thrombosis in rats were reported. Intravenous administration of GL caused a dose-dependent reduction in thrombus size on a venous thrombosis model that combines stasis and hypercoagulability. It was observed that GL doses of 180 mg/kg body weight produced 93% decrease on thrombus weight. GL doses above 90 mg/kg caused significant hemorrhagic effect. In contrast with heparin, GL did not potentiate the inhibitory activity of antithrombin III or heparin cofactor II towards thrombin. Altogether, data indicate that glycyrrhizin is an effective thrombin inhibitor in vivo, which may account for its other known pharmacological properties (Mendes- Silva *et al.*, 2003).

The effects of *G. glabra*, was studied on learning and memory. The elevated plus-maze and passive avoidance paradigm were employed to evaluate learning and memory parameters. Three doses (75, 150, and 300 mg/kg p.o.) of aqueous extract of Glycyrrhiza glabra were administered for 7 successive days in separate groups of mice. The dose of 150 mg/kg of the aqueous extract of licorice significantly improved learning and memory of mice. Furthermore, this dose reversed the amnesia induced by diazepam (1 mg/kg i.p.), scopolamine (0.4 mg/kg i.p.), and ethanol (1 g/kg i.p.). Anti-inflammatory and antioxidant properties of licorice may be contributing favorably to the memory enhancement effect. Since scopolamine-induced amnesia was reversed by licorice, it is possible that the beneficial effect on learning and memory may be because of facilitation of cholinergic transmission in brain. Licorice root has shown promise as a memory enhancer in both exteroceptive and interoceptive behavioral models of memory (Parle *et al.*, 2004).
The effect of licorice compounds, isoflavan and isoflavene groups on serotonin re-uptake of serotonin was studied and compared the results with the effect of other known phytoestrogens like genistein and daidzein. The results demonstrated that the isoflavans glabridin and 4'-O-methylglabridin (4'-OMeG) and the isoflavene glabrene inhibited serotonin re-uptake, whereas resorcinol, the isoflavan 2'-O-methylglabridin (2'-OMeG), and the isoflavones genistein and daidzein were inactive. This study concluded that several licorice isoflavans are unique phytoestrogens, which like estradiol, affects the serotonergic system and inhibits serotonin re-uptake and, thus, potentially may be beneficial for mild to moderate depression in pre- and postmenopausal women (Ofir et al., 2003).

3.12 Pharmacokinetic studies using *Glycyrrhiza glabra*

The pharmacokinetics of the major compound Glycyrrhizic acid was studied. When *Glycyrrhiza glabra* is orally administered, the major constituent Glycyrrhizic acid gets hydrolyzed into glycyrrhetic acid by intestinal bacteria possessing β-glucuronidase (Hattori et al., 1985; Akao et al., 1991). Glycyrrhizic acid is a potent inhibitor of 11-β-hydroxysteroid dehydrogenase. After oral dosing, Glycyrrhetic acid is rapidly absorbed and transported via carrier molecule to the liver. In liver, it is metabolized into glucuronide and sulphate conjugates, which are subsequently rehydrolysed to Glycyrrhetic acid. Glycyrrhetic acid is then reabsorbed, resulting in delay in terminal clearance from plasma (Ploeger et al., 2001). After oral administration of 100mg glycyrrhizin in healthy volunteers, no glycyrrhizin was found in the plasma, but glycyrrhetic acid was found at 200ng/mL. In 24 hour period after oral administration, glycyrrhizin was found in urine suggesting that it is partly absorbed as an intact molecule (Yamamura et al., 1992).

The pharmacokinetic and pharmacodynamic properties of liquiritin apioside, a main antitussive component of *Glycyrrhizae radix* (licorice) was studied with regard to its antitussive effect in guinea pigs. The results suggest that *G. radix* (licorice) may produce a
persistent antitussive effect, and that liquiritin apioside plays an important role in the earlier phase, while liquiritigenin, which is a metabolite of liquiritin apioside and liquiritin, plays an important role in the late phase (Kamei et al., 2005).

The effect of licorice on androgen metabolism was investigated in nine healthy women 22-26 years old, in the luteal phase of the cycle. They were given 3.5 g of a commercial preparation of licorice (containing 7.6% W.W. of glycyrrhizic acid) daily for two cycles. Licorice can reduce serum testosterone probably due to the block of 17-hydroxysteroid dehydrogenase and 17-20 lyase. Licorice could be considered an adjuvant therapy of hirsutism and polycystic ovary syndrome (Armanini et al., 2004).

The effects of hydrophobic flavonoids from licorice on abdominal fat accumulation and blood glucose level was investigated in obese diabetic mice. Licorice flavonoid oil was prepared by further extracting licorice ethanolic extract with medium-chain triglycerides (MCT), and adjusting the concentration of glabridin, the major flavonoid of licorice, to 1.2% in oil. Mice aged 6 weeks were assigned to 5 groups, and fed a high-fat diet containing 0 (control), 0.5%, 1%, or 2% licorice flavonoid oil, or 0.5% conjugated linoleic acid (CLA) for 4 weeks. Compared with the control, body weight gain and weights of abdominal adipose tissues were suppressed by feeding the diet containing 2% licorice flavonoid oil. These results indicate that licorice hydrophobic flavonoids have abdominal fat-lowering and hypoglycemic effects, possibly mediated via activation of peroxisome proliferator-activated receptor-gamma (Nakagawa et al., 2004).

The active principle of *Glycyrrhiza glabra*, the glycyrrhetinic acid, is responsible for sodium retention and hypertension. The effect of licorice on the reduction of body fat mass in healthy subjects was studied. Normal-weight subjects, 15 in number (7 males, age 22-26 yr, and 8 females, age 21-26 yr), were asked to consume 3.5 g a day of a commercial preparation of licorice for 2 months. Body fat mass and extracellular water (ECW) were
measured. Body mass index (BMI) did not change. ECW increased. Licorice was able to reduce body fat mass and to suppress aldosterone, without any change in BMI. Licorice can reduce fat by inhibiting 11beta-hydroxysteroid dehydrogenase Type 1 at the level of fat cells (Armanini et al., 2003).

The anti-atherogenic effects of licorice-root extract consumption in moderately hypercholesterolemic patients was analyzed. Supplementation of licorice root extract (0.1 g/d) to patients for 1 mo was followed by an additional 1 mo of placebo consumption. Licorice consumption reduced patients' plasma susceptibility to oxidation (by 19%); increased resistance of plasma LDL against three major atherogenic modifications: oxidation (by 55%), aggregation (by 28%), and retention, estimated as chondroitin sulfate binding ability (by 25%); reduced plasma cholesterol levels (by 5%), which was due to a 9% reduction in plasma LDL cholesterol levels; and reduced (by 14%) plasma triacylglycerol levels. After the 1 mo of placebo consumption, these parameters reversed toward baseline levels. Licorice extract supplementation also reduced systolic blood pressure by 10%, which was sustained during the placebo consumption. Dietary consumption of licorice-root extract by hypercholesterolemic patients may act as a moderate hypocholesterolemic nutrient and a potent antioxidant agent and, hence against cardiovascular disease (Fuhrman et al., 2002).

3.13 Antimicrobial activity

β-Glycyrrhetinic acid isolated from Glycyrrhiza glabra had an antibacterial activity of 7.6 and 12.5 µg ml⁻¹ against Bacillus subtilis and Staphylococcus epidermidis without causing hemolysis of human erythrocytes, whereas it was not inhibitory against Escherichia coli, Proteus vulgaris and various fungi. Confocal microscopy showed that β-glycyrrhetinic acid was located within the bacteria but had not caused membrane
disruption. It then inhibited synthesis of DNA, RNA and protein (Hyung Keun Kim et al., 2002).

The acne-therapeutic effects of Oriental herb extracts were investigated in terms of antichemotactic effect on polymorphonuclear leucocytes, antilipogenic actions, antibacterial activity against Propionibacterium acnes and resistance induction potency in the bacteria. The ethanol extract (0.01%) of Angelica dahurica markedly suppressed neutrophil chemotaxis, comparable to the effect of erythromycin (0.01%), whereas a strong antilipogenic effect was obtained with rhizoma coptidis (Coptis chinensis) extract (0.01%), leading to a higher efficacy than that of retinoic acid (0.01%). Interestingly, only Glycyrrhiza glabra showed a remarkable antibacterial activity against P. acnes, resulting in negligible induction of resistance, in comparison with a marked development of resistance in the bacteria treated with erythromycin. We suggest that an appropriate formulation containing A. dahurica, rhizoma coptidis and G. glabra could be helpful for the prevention and treatment of acne lesions (Nam et al., 2003).

In the course of screening for antifungal compounds from various plant material, licorice (Glycyrrhiza glabra) extracts with 80% methanol (oil-based extract of licorice; OEL) was found to have high fungicidal effect against Arthrinium sacchari M001 and Chaetomium funicola M002, and its active compound was identified as glabridin (3-(2’, 4’-dihydroxyphenyl)-8-dimethylpyrano [8, 7-e] chroman). OEL was effective against not only filamentous fungi but also some bacteria, especially thermo-resistant bacilli such as genera of Bacillus and Alicyclobacillus. Furthermore OEL reduced microorganism contamination in polyethyleneterephthalate (PET) bottled tea based beverages. These results indicate that OEL has potential commercial applications for the prevention of beverage and food spoilage due to microorganisms (Hiroshi and Jun, 2002).
Candida species represents a very minor component of the normal oral flora. Being an opportunistic microorganism, Candida sp. is able to transit from a harmless commensal organism to a pathogen (Himratul-Aznita et al., 2011). The emergence of higher resistance level of C. albicans and the non-Candida albicans (NAC) species towards many commonly prescribed antifungal agents (Nguyen et al., 1996) has become a great concern to society.

Stronger Neo-Minophagen C (SNMC) is a glycyrrhizin-containing preparation that is approved in Japan for the treatment of chronic hepatic diseases and is marketed in Japan, China, Korea, Taiwan, and India. The neuroprotective effects of SNMC in the postischemic rat brain after middle cerebral artery occlusion (MCAO) was studied. SNMC was used as 1 ml/kg, which is within the dose range used for the treatment of patients with chronic hepatic disease. The administration of SNMC intravenously at 30 minutes before or 30 minutes and 3 hours after MCAO (60 minutes) reduces mean infarct volumes to 27.0 ± 4.2%, 37.1 ± 12.4%, and 67.8 ± 5.8% of that of untreated controls, respectively. This neuroprotective effect is accompanied by improvements in motor impairment and neurological deficits. The administration of SNMC suppresses the microglia activation and neutrophil infiltration in the postischemic brain. In addition, SNMC suppresses lipopolysaccharide-induced nitrite production and proinflammatory cytokine induction in a microglia cell line, BV2. This indicates that the neuroprotective effect of SNMC might be due, at least in part, to an anti-inflammatory effect. Interestingly, SNMC shows significantly higher neuroprotective potency compared to an equivalent dose of pure glycyrrhizin, in terms of reducing infarct volume and improving neurological deficits. The results indicate that SNMC, a glycyrrhizin-containing preparation developed for chronic liver disease, has a marked neuroprotective function in the postischemic brain via its anti-inflammatory effects (Hiroshi and Jun, 2002).
3.14 Characterization Studies

The combination of the hyphenated techniques LC–MS and LC–SPE–NMR constitutes a powerful platform for the rapid isolation and identification of minor components from natural sources (Ref). Here, large scale multi-targeted metabolic profiling and fingerprinting techniques were utilized to help gain a broader insight into Glycyrrhiza species chemical composition. UV, MS and NMR spectra of extracted components were connected with NMR, MS, and multivariate analyses data from Glycyrrhiza glabra, Glycyrrhiza uralensis, Glycyrrhiza inflata and Glycyrrhiza echinata. Major peaks in (1)H NMR and MS spectra contributing to the discrimination among species were assigned as those of glycyrrhizin, 4-hydroxyphenyl acetic acid, and glycosidic conjugates of liquiritigenin/isoliquiritigenin. Primary metabolites profiling using GC-MS revealed the presence of cadaverine, an amino acid, exclusively found in G. inflata roots. Both LC-MS and NMR were found effective techniques in sample classification based on genetic and or geographical origin as revealed from derived PCA analysis (Farag et al., 2012).

The different species of Glycyrrhiza were analyzed by 1H NMR-based metabolomics technique. Partial least squares discriminant analysis (PLS-DA) was used as the multivariate statistical analysis of the 1H NMR data sets. There was a clear separation between various Glycyrrhiza species in the PLS-DA derived score plots. The PLS-DA model was validated, and the key metabolites contributing to the separation in the score plots of various Glycyrrhiza species were lactic acid, alanine, arginine, proline, malic acid, asparagine, choline, glycine, glucose, sucrose, 4- hydroxyphenylacetic acid, and formic acid. The compounds present at relatively high levels were glucose, and 4-hydroxyphenylacetic acid in G. glabra; lactic acid, alanine, and proline in G. inflata; and arginine, malic acid, and sucrose in G. uralensis. This is the first study to perform the global
metabolomic profiling and differentiation of *Glycyrrhiza* species using 1H NMR and multivariate statistical analysis (Yang *et al*., 2010) no back ref.

Investigations were carried out to identify the nature of the minor triterpenoids in liquorice (*G. glabra*) roots grown in Egypt. Five triterpenoids which had not been isolated previously from Egyptian sources of the root were determined. These were (i) 11-desoxoglycyrrhetic acid acetate methyl ester, (ii) 24-acetoxy-11-desoxoglycyrrhetic acid acetate methyl ester (iii) 11-desoxoglабrolide acetate, (iv) glabrolide acetate and (v) 3β-acetyl-18β-hydroxy-11-keto-olean-12-en-30-oic acid 30,18β-lactone. It is claimed that the fifth triterpenoid and its saponification product had not been extracted from *G. glabra* roots before the structural formulae and stereochemical configuration of the triterpenoids were determined using NMR (Elgamal *et al*., 1990)

3.15 Herbal Mouthwashes

Prevention of oral diseases is easier than a cure. The widespread use of mouthwashes as an aid to oral hygiene is a relatively recent phenomenon in the developing countries of the world. Development work on the mouthwashes has been done mostly by the manufacturers, and the little work that has been done relates to the individual ingredients they contain rather than to their complete formulations (Granby and Saldanha, 1984; Mat Ludin and Md Radzi, 2001; Tanzer *et al*., 1979).

Several researches have been carried out using different plant extracts. In one such study, the persica mouthwash could not inhibit the *Streptococcus mutans* of the oral cavity. Therefore, dental carries cannot be prevented (Jajrarm *et al*., 2009). Only few brands such as Listerine, Macleans, Colgate, Smokers and Signals are currently mentioning alcohol in the ingredients with even fewer brands mentioning the percentage of alcohol in the product (Wasif Haq *et al*., 2009).
The antibacterial activity of rosemary and sage against *Escherichia coli*, *Salmonella typhi*, *S. enteritidis* and *Shigella sonei* were studied by Bozin et al., 2007. Clove, cinnamon, Bishop’s weed, chilli, horseradish, cumin, tamarind, black cumin, pomegranate seeds, nutmeg, garlic, onion, tejpat, celery, cambodge were some of the plant parts tested against *Bacillus subtilis*, *Eschericia coli* and *Saccharomyces cerevisiae*. These plants showed potent antimicrobial activities (De et al., 1999).

Essential oils have been proved to exert antibacterial, antifungal, antiviral, and antioxidant properties but the mechanism of action is often not fully elucidated (Schelz et al., 2006). Essential oils based mouthwash has proved its value over years for the treatment of inflammatory conditions affecting the oral cavity and pharynx. This mouthwash mainly contains sage oil (*Oleum salviae*), eucalyptus oil (*Oleum eucalypti*), cinnamon oil (*Oleum cinnamoni*), fennel oil (*Oleum foeniculi*), aniseed oil (*Oleum anisi*), peppermint oil (menthol) and chlorophyllin (Graf, 2006).

Essential oils, namely Cinnamon, Clove, Lavender, Rose, Eucalyptus, Lemon, Menthol, Tea tree oil, Mustered and Rosemary oils were screened for potential antibacterial activity against medicinally important bacterial strains, which are present in dental plaques, namely *Streptococcus mutans* (MTCC 890), *Streptococcus mutans* (MTCC 497), *Streptococcus salivarius*, *Streptococcus mitis*, *Lactobacillus fermentum*, *Streptococcus anginosus*, *Streptococcus gordonii*, *Lactobacillus acidophilus*, and *Staphylococcus aureus*. The antibacterial activity was determined by agar well diffusion method. Out of ten essential oils tested, nine oils showed antibacterial activity against one or more strains. Cinnamon oil, Clove oil, Lavender oil, Rose oil, Eucalyptus oil, Lemon oil, Menthol, Tea tree oil and Rosemary oils exhibited significant inhibitory effect. These can be used to discover bioactive natural products, which are reduce the oral bacteria serve as leads in the development of new pharmaceuticals that address unmet therapeutic needs in oral medicine.
Majority of the oils showed antibacterial activity against the tested strains (Koteswara Rao et al., 2008).

Essential oil is more or less volatile material isolated from an odoriferous plant of a single botanical species, commonly extracted by steam distillation (Sattar et al., 1992). An oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. Essential oil differs from the fatty or fixed oils both in composition and properties. Essential oils have no fixed structure as they are the mixture of different components including alcohols, phenols, aldehydes, ketones, acids, terpenes, hydrocarbons and esters etc (Kothari et al., 2005). Medical applications of these oils range from skin treatments to remedies for cancer (Prakash et al., 2005).

In a study, menthol inhibited the growth of all tested bacteria except P. aeruginosa and observed good antifungal activity against the yeast C. albicans. The zones of inhibition ranged from 10.0–25.3 mm and 7.1–18.5 mm in diameter against C. albicans using the disc diffusion method. Furthermore, MIC values ranged from 15.6–31.2 μg/ml against tested bacteria and 125.0 μg/ml against C. albicans (Firas, 2009). Terpenenes or terpenoids have been previously reported to be active against bacteria (Ahmed et al., 1993; Amaral et al., 1998), fungi (Harrigan et al., 1993; Rana et al., 1997), viruses (Hasegawa et al., 1994; Xu et al., 1996), and protozoa (Vishwakarma, 1990; Ghoshal et al., 1996). The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Cowan, 1999).

3.16 Glycoside, the secondary metabolite

Cyanidin and its glycosides (Cy and Cyg) have been indicated as promising candidates as dietary compounds with a potential role in human health. They are the largest class of water-soluble compounds in plants, where they are responsible for the brilliant color (red, orange, blue) of fruits and flowers. As natural compounds of several foods such
as vegetables, fruits and red wines, they are estimated to be widely ingested by humans. This paper, basing on the data previously reviewed in 2002, focuses on the findings regarding human and animal studies on Cy and Cyg absorption and metabolism, antioxidant activity and biological properties, with particular attention to anticarcinogenic activity, vasoprotective, anti-inflammatory, anti-obesity and anti-diabetes effects. It is concluded that although Cy and Cyg bioavailability is low, further investigations are necessary because some important metabolites may still not have been identified. Literature data on antioxidant activity and biological properties, however, widely confirm Cy and Cyg as dietary compounds with a potential beneficial role in human health (Galvano, 2007).

3.17 DNA binding ability of various compounds

The detection of nucleic acids by fluorescent dyes such as SYBR Green I (SG) has become increasingly important for a variety of analytic and diagnostic applications (Vitzthum and Bernhagen, 2002). Since its introduction in the early 1990s (Haugland 2001), SG has been applied successfully in the detection of nucleic acids in gels (Schneeberger et al., 1995; Jin et al., 1996; Kiltie and Ryan, 1997; Diggle et al., 2003) in solution (Vitzthum et al., 1999; Rengarajan et al., 2002). Agarose (1%, w/v) gel electrophoresis was performed. Experiments were carried out under light protection. DNA samples were incubated for ~10 min with SG before loading the gels. When necessary, gels were stained with Ethidium Bromide (0.5 μg/ml TAE buffer) for 0.5 h and destained for 1 h in TAE buffer to check whether alterations in SG fluorescence intensities were due to inadequate loading of the gel. Imaging was carried out with the IDA Gel Documentation System and the AIDA version 2.0 software from Raytest Isotopengerätebau. Fluorescence excitation was performed at 312 nm. Fluorescence emission was detected using an EtBr or a SG gel stain photographic filter purchased from Molecular Probes. The two positive
charges of SG are likely to contribute to the high binding affinity for dsDNA (Zipper et al., 2004).

Table 6. Molecular Weights of Protein Standards for Polyacrylamide Gel Electrophoresis

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome c</td>
<td>11,700</td>
</tr>
<tr>
<td>β-Lactalbumin</td>
<td>14,200</td>
</tr>
<tr>
<td>Lysozyme (hen egg white)</td>
<td>14,300</td>
</tr>
<tr>
<td>Myoglobin (sperm whale)</td>
<td>16,800</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>18,400</td>
</tr>
<tr>
<td>Trypsin inhibitor (soybean)</td>
<td>20,100</td>
</tr>
<tr>
<td>Trypsinogen, PMSF treated</td>
<td>24,000</td>
</tr>
<tr>
<td>Carbonic anhydrase (bovine erythrocytes)</td>
<td>29,000</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle)</td>
<td>36,000</td>
</tr>
<tr>
<td>Lactate dehydrogenase (porcine heart)</td>
<td>36,000</td>
</tr>
<tr>
<td>Aldolase</td>
<td>40,000</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>45,000</td>
</tr>
<tr>
<td>Catalase</td>
<td>57,000</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>66,000</td>
</tr>
<tr>
<td>Phosphorylase b (rabbit muscle)</td>
<td>97,400</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>116,000</td>
</tr>
<tr>
<td>RNA polymerase, E. coli</td>
<td>160,000</td>
</tr>
<tr>
<td>Myosin, heavy chain (rabbit muscle)</td>
<td>205,000</td>
</tr>
</tbody>
</table>

3.18 Replication of E.coli chromosome

DNA replication of the E. coli chromosome begins at a single origin of replication (oriC) and proceeds bidirectionally to a termination site located approximately halfway around the circular chromosome. During replication, the DNA strands of the double helix
must be both unwound and separated. DNA replication is initiated when a protein encoded by the gene \textit{dnaA} binds repetitive 9-mer sequences at the origin. Subsequently, \textbf{helicases} specified by \textit{dnaB} and inhibitory proteins encoded by \textit{dnaC} bind repetitive 13-mer sequences. As helicase progresses 5´ to 3´, dissociation of protein DnaC allows the helicase to unwind the DNA. The unwinding produces positive superhelical turns in the rest of the DNA, making it energetically favorable to continue unwinding the strands. To unwind the DNA, positive superhelical turns have to be removed by cutting the DNA and allowing it to relax or by introducing negative superhelical turns to compensate for the positive ones. The introduction of negative superhelical turns requires energy and an enzyme called \textbf{DNA gyrase} (a topo- isomerase). DNA gyrase is an enzyme that can both remove positive supercoils or introduce negative supercoils into the DNA and thereby make strand separation energetically more favorable. Presumably the DNA gyrase binds ahead of the unwound DNA during replication. \textbf{Single-stranded binding proteins} (SSBPs) act to temporarily stabilize the unwound state (Stansfield, et al., 2003).

\textbf{3.19 Toxicity studies using Earthworm as a model}

One commonly used test organism for soil toxicity is the earthworm \textit{Eisenia fetida}. It was used to test common metal toxicants in a two week lethality test. The American Society for testing and materials (ASTM) standard guide for the \textit{E.fetida} soil toxicity test mention the Neuhauser procedure as one acceptable method of soil toxicity testing (ASTM 1995, 1998) no back ref.

The herbal plant \textit{Glycyrrhiza glabra} showed a novel hepatoprotective effects against DIC–induced hepatotoxicity in rats. Liver toxicity may be caused due to treatment with therapeutic agents. Diclofenac (DIC) is a widely used anti-inflammatory drug that causes liver toxicity. This DIC-induced hepatotoxicity was found to be associated with oxidative damage. The extracts of herbal plants namely \textit{Curcuma longa} (CL), \textit{Glycyrrhiza glabra}...
*Glycyrrhiza glabra* L (GL) and *Moringa oleifera Lam* (MO) were used in the study. They all showed a novel hepatoprotective effects against DIC–induced hepatotoxicity in rats. These results suggest that CL, GL, and MO inhibit DIC-induced hepatotoxicity and might serve as a novel combination chemotherapeutic agent with DIC to limit its free radical-induced organ injury (Hamza, 2007).

The cerebroprotective effect of the aqueous extract of the roots of *Glycyrrhiza glabra* Linn. (250 and 500 mg/kg) in hypoxic rats was evaluated. Hypoxia was induced by providing sodium nitrite drinking water to rats for 14 days. Extract at the tested doses promoted the locomotor activity and spatial behavior significantly, which was impaired in hypoxic rats. The extract administration restored the decreased levels of brain enzymes such as glutamate and dopamine and decreased acetylcholinesterase (AChE) activity significantly. Levels of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase were reduced due to hypoxia and were restored to near normalcy by administration of ethanol extract of *G. glabra*. Increased lipid peroxidation in hypoxic rats was also restored significantly by extract treatment. The ethanol extract of *G. glabra* possess a cerebroprotective effect in hypoxic rats, which may be mediated by its antioxidant effects.

The plant *Glycyrrhiza glabra* roots were evaluated, toxicologically and pharmacologically, for its antiulcer properties to develop cost effective and safe herbal drug with least side effects. In antiulcerogenic assay *G. glabra* was tested and compared with Cimetidine as positive control and physiological saline as negative control, using standard method. Ethanol induced ulcers were developed in albino rats and treated for 30 days. The ulcer indices were measured after 24 hours, 15 days and 30 days. Comparison shows that *G. glabra* possesses a very significant antiulcerogenic activity i.e. 77.7% after 15 days and
90% after 30 days of therapy. The results suggest that *G. glabra* could be a good source of alternative medicine for ulcer therapy (Fatima *et al*., 2008).

Every humans vary in their resistance/ susceptibility to various licorice preparations. So it is difficult to predict a dose appropriate for all individuals. Therefore a daily oral intake of 1- 10mg of glycyrrhizin, which corresponds to 1-5g licorice has been estimated to be a safe dose for most healthy adults (Walker 1994). High doses of licorice when used daily over a prolonged period can cause a fluid imbalance in the body, involving salt potassium, and water metabolism. Licorice associated hypertension is thought to be due to increased renal sodium retention (Uum, 2005).

### 3.20 Histological studies to analyze the internal anatomy

The wound healing activity of *Glycyrrhiza glabra* was improved by ensuring the physicochemical compatibility Glycyrrhiza glabra root Extract impregnated collagen and crosslinked collagen scaffolds (GGEICDS & GGEECCDS). This was formulated using different concentrations. The formulated scaffolds were subjected to physical, biochemical and histopathological examinations. Wound healing studies on Male Wister Rats were performed for a period of 7 days. When examined histopathologically, more production in the collagen content was clearly observed in 10% w/v GGEICDS treated groups which resulted in the epithelial gap reduction. The more count of fibroblast's (62 and 64 in 100µ in the wound healing process) in 10% GGEICDS and GGEECCDS treated groups was observed (Kishore Babu *et al*., 2011).

Administration of Diclofenac (DIC) at 150 mg/kg developed acute hepatic damage, which can be noted by increased serum alanine aminotransferase (ALT) activity and histopathological changes. In addition, DIC treatment resulted in an increase in the hepatic malonaldehyde level and depletion in total antioxidant capacity, reduced glutathione content, catalase, and superoxide dismutase activities. Treatment with herbal extracts of
Glycyrrhiza glabra for 30 days before DIC treatment significantly ameliorated the indices of hepatotoxicity induced by DIC. In addition, the herb alleviated DIC-induced oxidative changes in liver. Thus *Glycyrrhiza glabra* showed a novel hepatoprotective effects (Hamza, 2007).

Histopathological studies were carried out in rabbits to determine the effect of *Glycyrrhiza glabra* extract on blood lipids and atherosclerosis in rabbits fed with high cholesterol diet. Fifteen male rabbits were randomly divided into three groups (normal diet group, highcholesterol diet (1% cholesterol) and a group which received high-cholesterol diet supplemented with *Glycyrrhiza glabra* extract (50 mg/kg body weight every other day). The concentration of Total cholesterol (TC), LDL cholesterol, triglycerides (TG) and HDL cholesterol was determined in rabbits at the start of the experiment, and at the end of the first and second month of the study. At the end of the experimental period the aorta was removed for assessment of atherosclerotic plaques. Results show that *Glycyrrhiza glabra* significantly decreases TC, LDL and TG levels and increase HDL and lessens atherosclerotic lesion in aorta. Hence Glycyrrhiza glabra extract can effectively prevent the progress of atherosclerosis. This is likely due to the effect of *Glycyrrhiza glabra* on plasma lipoproteins and its antioxidant and anti-inflammatory properties (Uum, 2005).

The protective role of *Glycyrrhiza glabra* against acetaminophen (paracetamol) was assessed in mice histophysiologically. Histology was carried out to test the pathogenicity in mice. On day 9 after treating with various concentrations of acetaminophen, mice were sacrificed and liver and kidney were taken for the study. The tissues were fixed using Bouins fluid and stained using Hematoxylin – eosin (Anjali Sharma and Rathore, 2011).