4 REVIEW OF THE LITERATURE

4.1 Osteoporosis

Osteoporosis is a socio-economic health problem which leads to an increased risk of fractures. It is a metabolic disorder, characterized by decreased bone mineral density (BMD) and bone mass with micro architectural deterioration of bone structure, leading to enhanced bone fragility. WHO defines osteoporosis as BMD of subjects with a T-score at or below -2.5 standard deviation, below the peak adult bone mass. (33)

According to National Health and Nutrition Examination survey (NHANES III) an estimated 14 million American women over age 50 years are affected by low density at the hip. As per World Health Organization (WHO) 70% of women over the age 80 years have osteoporosis. This silently progressing metabolic disease is widely prevalent in India and osteoporotic fractures are a common cause of morbidity and mortality in adult men and women. The Progressive decrease in bone mass leads to an increased susceptibility to fractures mainly vertebral and hip fractures. (34, 35)

4.1.1 Types of osteoporosis

Osteoporosis is characterized as primary and secondary.

1. Primary osteoporosis

It is a condition of reduced bone mass and fractures, found in menopausal women (postmenopausal osteoporosis) or in older men and women (senile osteoporosis).
Primary osteoporosis represents fundamentally different conditions i.e. type I and type II.

- Type I is the loss of trabecular bone due to estrogen deficiency also called postmenopausal osteoporosis
- Type II is the loss of cortical and trabecular bone in men and women as the end result of age-related bone loss. It reflects the composite influence of long term remodeling efficiency, adequacy of dietary calcium and vitamin D, intestinal mineral absorption, renal mineral handling, and parathyroid hormone secretion.

2. Secondary osteoporosis

It is refers to bone loss resulting from specific clinical disorders, such as thyrotoxicosis or hyperadrenocorticism. Osteoporosis can be precisely diagnosed by measuring, bone mineral density using dual energy X-ray absorptiometry (DEXA). Markers of bone resorption and formation will be useful future additions in determining responses to treatment and the disease prognosis.

Treatment relies on preventing bone resorption, falls resulting in fractures and controlling pain. Therapy includes calcium and vitamin D supplementation, hormone replacement therapy, alendronate and nasal calcitonin. Many medications including phytoestrogens are under investigation to build bone or prevent its loss.(36, 37)

4.1.2 Risk factors for osteoporosis

The normal bone formation requires calcium and phosphate. Insufficient calcium intake or poor absorption of calcium from the diet causes inadequate bone
formation. Excessive resorption of Calcium and phosphate from the bones also adds up to the weakening of the bones. Both situations can result in brittle and fragile bones that can break easily. The leading cause of osteoporosis is a lack of certain hormones, particularly estrogen in women and androgen in men. Other factors that contribute to bone loss include inadequate intake of calcium and vitamin D, lack of weight-bearing exercise, and possibly other age-related changes in endocrine functions in addition to lack of estrogen. (Table 4-1) (38)

Table 4-1 Risk factors for Osteoporosis

<table>
<thead>
<tr>
<th>Genetic</th>
<th>White or Asian ethnicity, family history of osteoporosis or fractures, small body frame (Tall, thin, low body mass index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life style</td>
<td>Minimum exercise, sedentary, smoking, excessive alcohol, minimal sun exposure.</td>
</tr>
<tr>
<td>Diet</td>
<td>Low calcium intake any time in life, lactose intolerance, high caffeine, phosphorous, animal protein intake, weight loss greater than 10% after 50 years of age, Anorexia nervosa, long term parenteral nutrition.</td>
</tr>
<tr>
<td>Obstetric/Gynaecology</td>
<td>Late menarche, early natural menopause, surgical menopause, oopharectomy without replacement therapy,amenorrhea associated with anorexia nervosa, medications or excessive exercise.</td>
</tr>
<tr>
<td>History</td>
<td></td>
</tr>
<tr>
<td>Chronic illness</td>
<td>Low bone mineral density, hyperthyroidism, Cushings syndrome, diabetes, altered GI or hepatobiliary functions, occult osteogenesis imperfect, mastocytosis, arthritis, haemolytic anaemia, thalassemia, Gastric surgery, stroke.</td>
</tr>
<tr>
<td>Medication</td>
<td>Drugs altering calcium absorption, excessive thyroid replacement, glucocorticoids, long term heparin, chronic lithium therapy, chemotherapy, anti-convulsants.</td>
</tr>
<tr>
<td>Fall related Conditions</td>
<td>Medications: anxiolytics, long acting benzodiazepines, physical disability, slow gait, difficult tandem walk, decreased visual acuity, poor depth perception, use of walking aids.</td>
</tr>
</tbody>
</table>

4.1.3 Pathophysiology

Bone is living tissue and its strength depends upon the normal functioning of 3 key bone cells: osteoclasts, osteoblasts, and osteocytes. Osteoclasts and osteoblasts compose the bone multicellular unit (BMU), where bone remodeling and reconstruction occur. At the BMU, a small packet of old or damaged bone tissue is removed by the osteoclast in a process known as bone resorption. Osteoclast and osteoblast functions are well coordinated or coupled. Since the time needed for
osteoclasts to resorb bone is short (weeks), while the time needed for osteoblasts to form bone is long (months), any process in adults that increases the rate of bone remodeling will result in a net loss of bone. (39)

**Figure 4-1 Pathophysiology of Osteoporosis**

Two Primary Mechanisms Promote Increased Osteoclastogenesis and Bone Resorption in the Absence of Estrogen. Under estrogen-deficient conditions, T cells produce elevated levels of proinflammatory cytokines including TNF-alpha, IL-1, and IL-6. These cytokines promote increased RANK L expression on osteoblasts and stromal cells, which leads to osteoclast differentiation in the presence of M-CSF. In addition, CCR2 expression on osteoclast precursor cells is up regulated in the absence of estrogen. CCR2 signaling promotes the expression of RANK on these cells and increases their osteoclastogenic potential. Both mechanisms may contribute to the pathogenesis of postmenopausal osteoporosis.

**4.1.3.1 Bone Modelling and Remodelling**

Bone modelling and remodelling processes can work in two ways. First, they can work at the same time on different surfaces; the net effect is an increase in bone. This process is called modelling and is responsible for shaping or sculpting the skeleton during growth. Second, they can work together on the same area but at
different times to renew bone; the net effect is no change or a net loss. This process is called remodelling & is responsible for removing old bone and forming new bone. (40)

As a person ages, repeated strain or stress results in the development of micro damage. Accumulation of micro damage can reduce the strength of bone, and therefore, remodeling is necessary to repair the damage. Any substantial decrease in the rate of remodeling may increase the risk of spontaneous fractures because the skeleton's ability to repair itself is decreased. (41)

4.1.3.2 Post menopausal Osteoporosis:

Postmenopausal osteoporosis is characterized by an increase in bone resorption relative to bone formation, in conjunction with an increased rate of bone turnover. A sharp decrease in ovarian estrogen production is the predominant cause of rapid, hormone-related bone loss during the first decade after menopause. In postmenopausal women estrogen deficiency mainly affects both trabecular and cortical bone. (42, 43) In post menopausal osteoporosis the estrogen deficiency induce the production of macrophage colony stimulating factor; various pro-inflammatory cytokines like IL-1, IL-6, TNF-α and activated T cells from bone marrow stromal cells. (44)

Estrogen deficiency upregulates the formation and activation of osteoclast which leads to cortical porosity and enlarged bone resorption at trabecular bone site and increase fragility of bone. It also increases apoptosis of osteoblasts that limits the net increase in bone formation process. (45, 46) Estrogen directly affects on bone remodeling process via the receptors ERα and ERβ present on Osteoblast and Osteoclast. (47) Estrogen inhibits the activation of bone remodeling, most likely via
the osteocytes, and also inhibits bone resorption, largely by direct actions on OCs, but also by modulation of OB/osteocyte ratio and T-cell regulation of OC formation and activity.

In addition to these direct effects on OCs, estrogen also appears to regulate OC formation and activity indirectly. Combined in vitro and in vivo studies have demonstrated that estrogen suppresses RANKL production by OBs and T and B cells and also increases production of the decoy receptor for RANKL, OPG. (20, 48) Estrogen inhibits the activation of bone remodeling by directly acting on receptor and inhibiting the bone resorption. (49)

4.1.4 Prevention and treatment of osteoporosis

A rational strategy to prevent osteoporosis in young adults should be to achieve normal peak bone mass by getting enough vitamin D and calcium (1000 mg daily) in their diet and by performing weight-bearing exercise such as walking or aerobics and maintaining normal body weight. Pharmacological agents used to manage osteoporosis act by decreasing the rate of bone resorption, thereby slowing the rate of bone loss, or by promoting bone formation. (36)

4.1.4.1 Antiresorptive agents:

• **Calcium:** The rationale for using supplemental calcium to protect bone varies with time of life. Controlled trials indicate that supplemental calcium promotes adolescent bone acquisition, but its impact on peak bone mass is not known. In elderly subjects, supplemental calcium suppresses bone turnover, improves BMD, and decreases the incidence of fracture. Numerous Ca$^{2+}$ salts such as carbonate, lactate, gluconate, phosphate, and citrate as well as hydroxyapatite are available; the most frequently prescribed being the carbonates. Traditional dosing of calcium is about 1000 mg/day. (36)
• **Vitamin D and its Analogues:** Modest supplementation with vitamin D (400-800 IU per day) may improve intestinal Ca\(^{2+}\) absorption, suppress bone remodelling and improve BMD in individuals with marginal or deficient vitamin D status. Supplemental vitamin D has been shown to reduce fracture incidence in two European trials. Calcitriol and another polar vitamin D metabolite, hydroxyl cholecalciferol are used frequently in Japan and other countries. A higher dose of calcitriol has a risk of hypercalciuria and hypercalcemia. (50)

• **Estrogen:** Overwhelming evidence confirms a major role for menopausal estrogen replacement in the conservation of bone and protection against osteoporotic fracture. Studies indicate that 17 β – estradiol acts on osteoblasts to decrease production of interleukin-6 and to up regulate the production of Osteoprotegerin (OPG), thereby interfering with recruitment of osteoclast precursors. As sole therapy, the minimum effective dose of estrogen for skeletal protection is 0.625 mg/day of conjugated equine estrogens (PREMARIN) or its equivalent. The optimal time to institute estrogen replacement is early menopause, when bone turnover accelerates, however, even for women beyond age 65, beneficial skeletal effects of estrogen are observed. (51) A large proportion of postmenopausal women do not use estrogen, in part because of the frequency of side effects (including breast tenderness and menstrual bleeding) and in part because of fear of an increased risk for breast cancer. Thus, initiation of estrogen therapy to elderly women must be individualized. (52)

• **Selective Estradiol Receptor Modulators (SERMs):** For women who are unable to take estrogen or choose not to, selective estrogen receptor modulators (SERMs) such as raloxifene (Evista) offer an alternative. Tamoxifen (Nolvadex), commonly used in the treatment of certain breast cancer, also inhibits bone
breakdown and preserves bone mass. Anti-estrogens, such as tamoxifen, raloxifene and droloxifen can have beneficial effects on bone and might reduce the incidence of breast cancer. Raloxifene significantly increases the bone mineral density in spine, hip and total body, and also decreases the risk of vertebral fracture. These drugs are prone to cause venous thrombosis and hot flushes. (36)

- **Bisphosphonates:** Bisphosphonates have emerged as the most effective drugs currently approved for prevention and treatment of osteoporosis. They have been shown in clinical trials to increase bone mass and decrease the rate of vertebral fractures. The recommended course is 10 mg/day for 3 years. One drawback of all bisphosphonates, including alendronate, is that they are poorly absorbed orally and must be taken long before or after any food or other medication. No unexpected long-term side effects have been reported to date. However nausea, dyspepsia, gastrointestinal ulcers are some of the common short term side effects. (52, 53)

**Alendronate:** Current data indicate that alendronate is an appropriate therapy for postmenopausal women with established osteoporosis who cannot or will not take estrogen. At present, alendronate is indicated for patients, not receiving estrogens, who have had vertebral crush fractures associated with low bone mass. (36)

**Risedronate:** This drug is used for the treatment and prevention of osteoporosis. Gastrointestinal upsets are the most common side effect. Women with severe kidney impairment should avoid this drug. (36)

**Etidronate:** This drug has been approved by the FDA for treatment of Paget’s disease, another bone condition. Yet doctors have been using this drug successfully to treat women with osteoporosis. (36)
• **Thiazide Diuretics:** Although not strictly antiresorptive, thiazide reduce urinary Ca\(^{2+}\) excretion and constrain bone loss in patients with hypercalciuria. Data suggest that they reduce hip fracture risk. Hydrochlorothiazide, 25 mg once or twice daily may achieve substantial reduction in calciuria. (36)

4.1.4.2 Bone Forming Agents

• **Androgen:** Testosterone replacement therapy increases BMD in hypogonadal men. Androgens also improve BMD in osteopathic women but is limited by virilizing side effects. Nandrolone decanoate (50 mg by injection every three weeks) increases peripheral and axial BMD without bothersome side effects in osteoporotic women. The androgenic progestin norethisterone acetate acts synergistically with estrogen to increase BMD in osteoporotic women. (50)

• **Parathyroid Hormone (PTH):** Several laboratories have examined the effects of PTH on BMD in patients with osteoporosis. The synthetic analog hPTH increased axial bone mineral, although effects on cortical bone were disappointing. Co-administration of hPTH with estrogen or synthetic androgen led to impressive gains in axial mineral without substantial gains in BMD in patients with glucocorticoid-associated osteoporosis. Phase III controlled clinical trials of PTH and its analogs are currently in progress. (50)

• **Phytoestrogens:** Phytoestrogens such as isoflavones (Genistein, Daidzein) and lignans (Enterodiol, Enterolactone) may have weak estrogenic or antiestrogenic activity. Thereby soya bean, soya flour, textured vegetable protein, tempeh and tofu rich in isoflavones and flax seed and some cereals, vegetables, legumes and fruits rich in lignans are given as nutritional supplements in the prevention of osteoporosis. Ipriflavone, a synthetic flavonoid derivative is found effective in preserving bone mass in several models of experimental osteoporosis. (50)
4.2 Animal models for Osteoporosis

Osteoporosis is well developed in rat model because in rat bone loss occurs is more prominent. In rat sexual maturity reaches at the age of 2.5 month, and their skeleton is considered mature after the age of 10 month. The skeletally mature rat is an appropriate animal model for studying postmenopausal and immobilization osteoporosis. (54, 55)

![Diagram](image)

**Figure 4-2 Rat Models for Osteoporosis**

Algorithm for the selection of experimental interventions to induce osteoporosis in the Laboratory rat

4.2.1 Dietary intervention

Deficiency of minerals occur due to Low Calcium diet and other minerals like Phosphorous, Magnesium, Zinc deficiency in diet causes the Osteoporosis because they are important in maintaining bone quality due to their role in the synthesis of
collagen and other bone proteins. Zinc (Zn) causes a reduction in osteoclastic activity, collagen synthesis and alkaline phosphatase activity and stimulates bone formation. Mg depletion results in hypocalcemia, low serum parathyroid hormone (PTH) and 1, 25(OH)2-vitamin D levels, as well as PTH and vitamin D resistance which may serve as mechanisms for the development of osteoporosis. (56) Phosphate deficiency, induced release of various inflammatory cytokines which is responsible for the bone loss. (57)

4.2.2 Immobilization induced osteoporosis

Immobilization induced osteoporosis in rat involved local as well as systemic immobilization. The local immobilization or disuse models are performed in one limb. The different disuse models differ in speed of bone loss depending upon the Regional Acceleratory Phenomenon (RAP), it constitutes a considerable acceleration of all normal tissue turnover processes. In the immobilization model, the bulk of bone loss occurs in the hind limbs, because they are the sites of greatest mechanical loading, but in general, the rate of bone loss is faster in cancellous than in cortical bone. Bones mainly trabecular bones with higher peak bone mass loose more bone than those with lower bone mass. (58)

Table 4-2 Effect of Immobilization osteoporosis on different bones in rat

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Suspension</th>
<th>Limb taping</th>
<th>Nerve resection</th>
<th>Tenotomy</th>
<th>Limb casting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>Hind limb</td>
<td>One hind limb</td>
<td>One hind limb</td>
<td>Lower hind limb; foot</td>
<td>One limb</td>
</tr>
<tr>
<td>Surgery</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Timeframe Responses</td>
<td>Short term &lt; 5 weeks</td>
<td>Long term</td>
<td>Long term</td>
<td>Short term</td>
<td>Long term</td>
</tr>
<tr>
<td>Cancellous bone loss</td>
<td>No</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Trabecular bone</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
</tbody>
</table>
### 4.2.3 Hormonal Intervention

Estrogen is an important hormone which regulates the bone remodeling process. These drugs include estrogen receptor antagonist, GnRH agonist, Aromatase inhibitor and Corticosteroids. (59)

#### 4.2.3.1 Aromatase inhibitors

In Peripheral tissues adrenal androgen is converted into estrogen after menopause. The Aromatase inhibitor inhibits the aromatase enzyme, responsible for this conversion and result in decrease concentration of estrogen, so many times the treatment with aromatase inhibitor might be expected to cause bone loss and increased fracture risk.

#### 4.2.3.2 Gonadotropin-releasing hormone agonist

The GnRH agonist down regulates the secretion of Luteinizing hormone (LH) and Follicle Stimulating Hormone (FSH), resulting in suppression of ovarian function and a corresponding decline in estrogen production. Bone loss is a well known consequence of hypogonadism. So, it follows that decreased BMD is a potential complication of GnRH agonist.

#### 4.2.3.3 Corticosteroid induced Osteoporosis

Corticosteroid leads to a reduction in osteoblast number and function by inhibition of replication and differentiation of cells of osteoblastic lineage, and enhanced apoptosis of osteoblasts and osteocytes. Glucocorticoid-induced

<table>
<thead>
<tr>
<th>Formation</th>
<th>Trabecular Resorption</th>
<th>No</th>
<th>Increase</th>
<th>Increase</th>
<th>Increase</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical Bone loss</td>
<td>No</td>
<td>Decrease</td>
<td>Decrease</td>
<td>-</td>
<td>Decrease</td>
<td></td>
</tr>
</tbody>
</table>

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Kadi Sarva Vishwavidyalaya

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K.B.I.P.E.R
osteoporosis is the most common cause of drug-induced osteoporosis, leading to fragility fractures. (60, 61)

4.2.4 Surgical Models

4.2.4.1 Gonadectomy

4.2.4.1.1 Ovariectomy (62, 63)

As per UCSF Rodent Anesthesia Guidelines, single midline skin incision on the back, Skin incision is approx. 10 mm in rat. The skin is separated from the underlying muscle before incising the muscle.

The ovary is gently pulled through the incision with a blunt forceps by grasping the fat pad surrounding it. A hemostat or similar is placed at the boundary between the oviduct and uterus, a ligature placed just below the haemostat (next to the uterus) and a cut is made just above the haemostat. Once the ovary and oviduct are removed, the haemostatis released and muscle layer is closed, usually with absorbable suture. Skin is closed with suture or wound clips.

4.2.4.1.2 Orchidectomy (62)

The animal is weighed and anesthetized as per UCSF Rodent Anesthesia Guidelines and made single transverse incision at the caudal abdomen, or a single midline incision on the scrotal sac.

Skin is separated from the muscle layer and then a similar transverse incision is made through muscle. Alternatively both testes are push down into the scrotum by gentle pressure on the abdomen and 5 mm incision is made through the scrotal skin and the underlying tunica. The testicular fat pad on the one side is pulled through the incision using a blunt forceps. haemostat is placed below the testes and epididymis across the testicular cord (contains blood vessels and vas deferens).
Then ligature (absorbable suture is preferred) is placed below the haemostat then removes the testes and epididymis with a scissors, Lastly the haemostat is released and makes sure that bleeding is not occur. The incision is closed in two layers with suture or wound clips.

4.2.4.1.3 Hypophysectomy (64)

Hypophysectomy is the surgical removal of the hypophysis (pituitary gland). A median skin incision is made 2.5 cm forward from the sternum. The large salivary glands and the maxillary lymph nodes are pushed to the side, the muscles over the trachea are divided at the midline, and slightly below the thyroid a hole is made in the trachea with a needle. A deep blunt dissection is made directly medial to the tendon of the left or right digastric muscle. Then, a needle with a diameter of 1.6 mm and with a blunted tip being attached to a glass cannula is introduced and the pituitary is removed by suction. (64)

4.2.4.1.4 Parathyroidectomy

The Parathyroids are generally located symmetrically in the neck in close proximity to the thyroid gland. (65) The Lateral approach involves dissecting along the medial border of the sterno-cleidomastoid muscle to the carotid sheath, and then medial to the sheath up to the thyroid region. The anterior approach involves medial mobilisation of the thyroid gland. This is used in cases of possible bilateral neck exploration. (66)

4.3 Ovariectomy induced post menopausal osteoporosis in rat

This model is most commonly used in osteoporosis research. After ovariectomy, there is a rapid and reproducible bone loss occurring due to estrogen deficiency and resultant increase in bone resorption. (67, 68)
4.3.1 Incision methods

There are different types of incision methods for removing ovaries like

1. Midline Skin incision
2. Two Dorsolateral incision
3. Transverse incision

4.3.1.1 Midline skin incision

The anesthetized animal’s dorsal mid-lumbar area is shaved and sterilized with alcohol. A 2-3 cm dorsal midline skin incision is made. Each ovary and part of the oviduct is removed with single cuts through the oviducts near the ovary. The ovary on the other side is removed in a similar manner, recover the animal & put in to individual cage under sterile aspetic area. (69)

![Figure 4-3 Midline Skin Incision](image)

4.3.1.2 Two Dorsolateral incision

In two dorsolateral incisions; one small incision (1.5 cm) is made through the skin and the muscle wall on each side of the backbone, in the dorsal aspect. Each ovary and part of the oviduct is removed with two cuts through the oviducts near the ovary.
4.3.1.3 Transverse incision

Transverse incision is made after placing an anesthetized animal on its dorsal surface. A small transverse peritoneal incision of 0.4–0.6 cm is made with surgical scalpel on the middle part of the abdomen. After peritoneal cavity is accessed, the adipose tissue is pulled away until the right uterine tube and the ovary surrounded by a variable amount of fat are identified. The ovary and associated fat are easily located and exteriorized by gentle retraction. The procedure is repeated for the left ovary through the same incision. After identifying the ovary and uterine horn, silk suture is performed around the area of the distal uterine horns, that is sectioned thereafter, and the ovaries are removed.

The uterine horn is returned to the peritoneal cavity after the removal of ovaries. The wound is closed in two layers (muscle and skin) using sterile sutures. The peritoneum and the muscle layers are sutured with one absorbable suture and the skin is suture with one non-absorbable suture. (68)
Figure 4-5 Transverse Incision

Transverse incision made on the middle part of abdomen slightly towards the right with a surgical scalpel blade. The transverse abdominal muscle is exposed after skin incision

4.3.2 Ovariectomy

After the muscle dissection, the peritoneal space and adipose tissue surrounding ovary are exposed. Thick black circles in figure 4-6 and 4-7 show the ovary surrounded by adipose tissue. Ligation at the distal uterine horn in order to completely remove the ovary, one at a time. The ovary surrounded by fat is completely removed using different techniques like using forceps, titanium clip and nylon ligature. (69)(figure 4-8)

Figure 4-6 Location of Ovaries
Figure 4-7 Removal of Ovaries

Figure 4-8 Ovary surrounded by adipose tissue cut using forceps, titanium clip and nylon ligature

4.3.3 Benefit of ovariectomised model over other model

Ovariectomised Rat rapidly loss ovarian hormones and have complete loss of trabecular bones, that resembles same like Post menopausal Osteoporosis. (70) In Post menopausal Osteoporosis, after ovariectomy female rat skeleton is more sensitive to the loss of ovarian hormones & exhibit most of the characteristics of human postmenopausal osteoporosis, so this model selected for study. (58)

4.3.4 Post operative care required

Povidone iodine is applied on the area to disinfect the skin after suturing. High degree of aseptic procedure is maintained throughout the operation. After
surgery, the rats are housed individually in polyurethane boxes for a period of one week to allow recovery and then re-grouped in their home cages. (68)

4.4 Review of herbal drugs selected for study

4.4.1 Arjuna bark

4.4.1.1 Botanical source

It consists of stem bark of *Terminalia arjuna*. It belongs to family Combretaceae

4.4.1.2 Common names (71)

Assam: Arjun

Beng. – Arjhan

Guj. – Sadado

Hin.- Arjuna

Kan. – Maddi, Bihimatti

Mar.- Sanmadat

Ori.- Arjuna

Punj. – Arjon

Tam. – Vellamatta, Marudam

Tel. - Yeramaddi

In Ayurveda, It is also known as Dhavala, Kakubha, Nadisarja, Veeravriksha, Partha, Indradru.

4.4.1.3 Chemical constituents

Major: Hydrolysable and condensed tannins, ellagic acid, arjunin, arjunic acid, arjunetin, arjunolitin, friedelin, terminoic acid, arjungenin, arjunglucoside I and II.

Others: β- sitosterol and flavone arjunolone. (71)
4.4.1.4 Traditional uses

It pacifies kapha with its astringent, light and dry qualities. It pacifies pitta with its cool properties. Because of its astringent properties, it powder or decoction is good for application on wounds. It helps in their healing. Its astringent nature make it useful in bleeding piles and diarrhea with blood. Biliary affections are relieved by this herb. It has general tonic effect in Cirrhosis of Liver. It is cardio-tonic thus providing nutrition to heart muscles and strengthening them. It normalizes the disturbed rhythms of heart and also reduces the heart rate. This is found to be helpful in edema as well. It reduces the clotting tendency of blood. It reduces the stress and nervousness of the heart. It helps in reversing the hardening of the blood vessels. It is found to be useful in cough (particularly sputum with haemoptysis) along with Vasa (*Adhatoda vasica*) leaves. So it could be used as adjuvant in tubercular cough.

It can be used with sugar, rice water and equal quantity of red sandalwood in hemoptysis. This helps to stop the blood and heal the ulcer. It is useful in urinary tract infections and reduces the burning micturition. Because of its diuretic action, it was found to be helpful in treating renal or urinary bladder stones. Spermatorrhea is relieved with the decoction of its bark and white sandal according to Sushruta. Harita also advised its decoction in gonorrhea. It relieves leucorrhea and excessive menstrual bleeding. Vagbhata advised it to be applied locally with honey for acne. It can be given as an adjuvant in chronic fevers, particularly tuberculosis. Prolonged use is good for obese persons, in chronic poisoning and general debility. With milk it is given in fractures and contusions with excessive ecchymosis as it promotes the union of fractures. (72)
4.4.1.5 Pharmacological actions reported

A number of previous studies have reported a wide range of pharmacological activities of *Terminalia arjuna*. (Table 4-3)
Table 4-3 Pharmacological actions of *Terminalia arjuna*

<table>
<thead>
<tr>
<th>PHARMACOLOGICAL ACTIVITY</th>
<th>EXTRACT USED</th>
<th>EXPERIMENTAL MODEL</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial</td>
<td>Different extract</td>
<td>Inhibition assay</td>
<td>(73, 74)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Different extract</td>
<td>Experimental animals induced with DLA (Dalton’s Lymphoma Ascites) tumour cells</td>
<td>(75)</td>
</tr>
<tr>
<td>Antifungal</td>
<td>Methanolic</td>
<td>Paper disc method</td>
<td>(76, 77)</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>Arjunolic acid Different extracts</td>
<td>alloxan induced diabetes in rats.</td>
<td>(77-80)</td>
</tr>
<tr>
<td>Antiacne</td>
<td>Flavanoid and tannin fraction</td>
<td><em>In vitro</em> antibacterial activity</td>
<td>(81)</td>
</tr>
<tr>
<td>Anthelmintic</td>
<td>Methanolic extract</td>
<td>Egg hatch test, larval development test and adult motility assay.</td>
<td>(82)</td>
</tr>
<tr>
<td>Wound healing</td>
<td>Ethanolic extract</td>
<td>Incision and excision wound models</td>
<td>(77, 83)</td>
</tr>
<tr>
<td>Cardioprotective</td>
<td>Aqueous extract</td>
<td>Isolated rabbit and frog heart</td>
<td>(77, 84-87)</td>
</tr>
<tr>
<td>Insecticidal property</td>
<td>Arjunolic acid</td>
<td>Inhibitory activity towards fourth instar larvae of <em>Spilarctia obliqua</em></td>
<td>(77)</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Ethanolic extract</td>
<td>Inhibits the enzyme cycloxygenase (COX) leading to inhibition of prostaglandin synthesis</td>
<td>(77)</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>methanol extract</td>
<td>Free radical scavenging activity</td>
<td>(88)</td>
</tr>
<tr>
<td>Antiasthmatic</td>
<td>Arjunolic acid and alcoholic extract</td>
<td>Mast cell stabilization activity <em>in vitro</em></td>
<td>(77, 89)</td>
</tr>
<tr>
<td>Gastroprotective effect</td>
<td>Methanolic extract</td>
<td>Diclofenac sodium induced gastric ulcer in rats</td>
<td>(90)</td>
</tr>
<tr>
<td>Decrease arsenic-induced toxicity</td>
<td>Arjunolic acid</td>
<td>Arsenic-induced cytotoxicity in isolated murine hepatocytes</td>
<td>(91)</td>
</tr>
<tr>
<td>Healing of bone fracture</td>
<td>Ethanolic extract</td>
<td>Experimentally fractured tibia of rats.</td>
<td>(28)</td>
</tr>
<tr>
<td>Increase Coronary flow</td>
<td>Aqueous extract</td>
<td>Isolated rabbit heart (Langendorff’s)</td>
<td>(92)</td>
</tr>
<tr>
<td>Hypotensive effects</td>
<td>Aqueous extract</td>
<td>Hypotensive effect in dogs</td>
<td>(93)</td>
</tr>
</tbody>
</table>
4.4.2 Acacia bark

4.4.2.1 Botanical source

It consists of dried stem bark of *Acacia arabica*. It belongs to family Mimosaceae.

4.4.2.2 Common names

Assam. – Babala
Beng. – Babla
Eng. – Indian gum, Arabic tree, Babula tree
Guj. – Baval, Kalobaval
Hind. – Babula, Babura, Kirkar
Kan. – Kari jali, Shameeruka, Karigobili, Pulai jali
Kash. – Sak
Mal. – Karuvelan, Velutha
Mar. – Babhul, Vabhula
Ori. – Babula, Badala
Punj. – Kikkar
Tam. – karuvelan, Karuvel
Tel. – Nellatumma, Thumma (94)

4.4.2.3 Chemical constituents

**Major:** Arabic acid.

**Others:** 6-O-(β-D-Glucopyranosyluronic acid)-D-galactose, 6-O-(4-O-methyl-β-D-glucopyranosyluronic acid)-D-galactose, 4-O-(α-D-glucopyranosyluronic acid)-D-galactose, 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose. (94)
4.4.2.4 Traditional use

Bark is used as astringent, acrid cooling, styptic, emollient, anthelmintic, aphrodisiac, diuretic, expectorant, emetic, nutritive, in hemorrhage, wound ulcers, leprosy, leucoderma, small pox, skin diseases, biliousness, burning sensation, toothache, leucoderma, dysentery and seminal weakness. The trunk bark is used for cold, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma. (95-101)

The bark contains a large quantity of tannin and is a powerful astringent; its decoction is largely used as a gargle and mouth wash in cancerous and syphilitic affections (102). Infusion of bark (11/2 ounces to one pint of water) is given in chronic diarrhoea and diabetes mellitus in doses of 11/2 to 2 ounces twice a day. The juice of the bark mixed with milk is dropped into the eye in conjunctivitis (102). Decoction of bark is largely used as an astringent douch in gonorrhoea, cystitis, vaginitis, leucorrhoea and prolapse of uterus. (103)

4.4.2.5 Pharmacological actions reported

A number of previous studies have reported a wide range of pharmacological activities of Acacia arabica. (Table 4-4)
Table 4-4 Pharmacological actions of *Acacia arabica*

<table>
<thead>
<tr>
<th>PHARMACOLOGICAL ACTIVITY</th>
<th>EXTRACT USED</th>
<th>EXPERIMENTAL MODEL</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti bacterial and antifungal</td>
<td>Stem bark extract</td>
<td>Agar diffusion method</td>
<td>(104)</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>Water extract</td>
<td>lipid peroxidation assay</td>
<td>(105)</td>
</tr>
<tr>
<td>Anti-mutagenic</td>
<td>Acetone extract</td>
<td>7,12-dimethylbenz(a)anthracene (DMBA) induced skin papillomagenesis in male swiss albino mice</td>
<td>(106)</td>
</tr>
<tr>
<td>Hepatoprotective</td>
<td>Methanolic</td>
<td>acetaminophen-induced hepatotoxicity</td>
<td>(107)</td>
</tr>
<tr>
<td>Antidiarrhoeal</td>
<td>petroleum ether, methanol and distilled water</td>
<td>Castor oil and magnesium sulphate induced diarrhoea and barium chloride induced peristalsis using Swiss albino mice</td>
<td>(108)</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>Methanolic</td>
<td>Streptozotocin induced diabetes in rat</td>
<td>(109)</td>
</tr>
<tr>
<td>Antiplasmodial</td>
<td>Ethyl acetate Methanolic</td>
<td>Plasmodium falciparum induced malaria in mice chloroquine sensitive strain of Plasmodium berghei induced malaria in mice</td>
<td>(110, 111)</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>Methanolic</td>
<td><em>in vitro</em> study on guinea pig paired atria</td>
<td>(112)</td>
</tr>
<tr>
<td>Antispasmodic</td>
<td>Aqueous</td>
<td><em>in vitro</em> study on rabbit jejunum</td>
<td>(113)</td>
</tr>
<tr>
<td>Antialzheimer</td>
<td>Methanolic</td>
<td>Acetylcholinesterase inhibition assay</td>
<td>(114)</td>
</tr>
</tbody>
</table>
4.4.3 Oleo-gum-resin of Guggul

4.4.3.1 Botanical source

It consists of dried gum of *Commiphora mukul* belonging to family Burseraceae.

4.4.3.2 Common names

Beng. - Guggul
Eng. – Indian bdellium tree
Guj. - Guggul
Hind. - Guggul
Kan. - Guggula
Mar. - Guggul
Tam. - Mahisaksi, Gukkulu
Tel. - Guggulu (115)

4.4.3.3 Chemical constituents

**Major:** Resin, Gum, Volatile oils consists of myrcene, dimyrcene, polymyrcene. Resin contains Z- guggulsterone, E- guggulsterone, Z- guggulsterol, guggulsterol I-V.

**Others:** 20- α-hydroxy-4-pregnen-3-one, 20- β-hydroxy-4-pregnen-3-one, 16-β-hydroxy-4, 17(20)Z-pregnadien-3-one and 16 α-hydroxy-4- pregnen-3-one, cembrene, mukulol. (115)

4.4.3.4 Traditional uses

Similar to another important Ayurvedic preparation called triphala, guggul is considered tridoshic, or balancing to all three doshas in the body. The three doshas or bodily humours of the body represent the foundation of traditional Ayurveda.
These are: kapha or the anabolic humour, watery humour; pitta or the catabolic, fiery humour; and vata, the air or nervous system humour. When all three humours are in balance, the result is health and wellness. When one or more are excess or deficient this represents imbalance or disease. Guggul stimulates pitta and thus enhances warmth, digestion, circulatory and reproductive processes. It also regulates vata (nerve force) and kapha (fluidic aspects).

It is considered the most important for the removal of "ama," toxic substances which accumulate as a result of sluggish digestion and circulation associated with a slowing of metabolism.

As an "ama"-resolving herb, guggul has a wide range of applications beginning with rheumatic and arthritic pains and obesity. In addition it treats sluggish liver, malaria, stimulates libido, nervous diseases, bronchial congestion, cardiac and circulatory problems, weak digestion, fractures, gynecological problems, leucorrhea, sterility, impotence, STDs (sexually transmitted diseases) and various skin diseases including acne and psoriasis.

Guggul has been used for over 3,000 years and is described in all of the classical Ayurvedic texts including the Sushruta Samhita (3rd to 4th centuries) where it is especially recommended for the treatment of rheumatic pains and obesity, as mentioned above. It is one of the most important rasayanas (herbal tonics) of Ayurveda where it is described as warm, dry, pungent-flavored, and aromatic with nutritive, lubricant, stimulant and digestion-enhancing properties. The Sushruta recommends guggul for a condition called medoroga (obesity). Current research substantiates its benefit for the treatment of elevated blood lipids and coronary and arterial plaque known as atherosclerosis. As a result, today standardized guggul
extracts are being approved for lowering elevated serum cholesterol and triglyceride levels in India.

The traditional properties of guggul are demulcent, aperient, carminative, antispasmodic and emmenagogue. On the mucus membranes, it serves as an astringent and antiseptic. Internally, its bitter principles stimulate appetite and relieve bloating and gas. Its oleo-resins are excreted through the skin, mucus membranes, and the urinary system, stimulating and disinfecting their secretions. It is also a uterine stimulant, making it useful for regulating menstruation but contraindicated during pregnancy. The warming, circulatory properties of guggul also serve as a potent aphrodisiac.

Guggul is warming and stimulates metabolism that is why it is one of the few botanicals that has been shown to treat hypothyroid conditions.

Guggul also serves as an antipyretic in reducing fever and reduces secretions from diseased surfaces of the body. As such it is excellent when used synergistically with other anti-inflammatory herbs such as tinospora (guduchi), echinacea and goldenseal (hydrastis).

Guggul can be given in large doses several times daily for laryngitis, bronchitis, pneumonia and whooping cough. The fumes of burning guggul can be inhaled for hay fever, acute and chronic nasal congestion, chronic laryngitis, chronic bronchitis and tuberculosis. A plaster of the powder applied to the pit of the stomach stops hiccough instantly.

Guggul, as with other resins, is excreted through the skin, mucus membranes and the kidneys. This makes it particularly useful for the urinary tract and for a wide number of skin diseases including acne and psoriasis. (116-118)
4.4.3.5 Pharmacological actions reported

*Commiphora mukul* is an important medicinal plant. More recently its use in cardioprotection and the treatment of nervous disorders has also been demonstrated. Studies also show that gum guggul is an efficacious and cost-effective treatment of thyroid disorders and infections. Ayurvedic herbs in combination with *C. mukul* have been marketed for the treatment of arthritis, obesity and related disorders. Several patents have been assigned to guggul for use in cosmetics and as a constituent of polyherbal formulation. Considering its commercial, as well as medicinal importance *C. mukul* can be advocated as a highly valuable medicinal plant. (Table 4-5)
Table 4-5 Pharmacological actions of *Commiphora mukul*

<table>
<thead>
<tr>
<th>PHARMACOLOGICAL ACTIVITY</th>
<th>EXTRACT USED</th>
<th>EXPERIMENTAL MODEL</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiinflammatory</td>
<td>Ethyl acetate extract</td>
<td>Croton oil-induced oedema of mice ears</td>
<td>(119) Otitis media. (120) Rheumatoid arthritis (121)</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>Guggulipid</td>
<td>Randomized, double-blind clinical study</td>
<td>(122)</td>
</tr>
<tr>
<td>Bone resorption</td>
<td>Guggulsterone</td>
<td>Suppression of monocyte differentiation into osteoclasts</td>
<td>(123)</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Guggulsterone</td>
<td>Intestinal epithelial cells (IEC) and on murine colitis-induced ex- perimental models</td>
<td>(124)</td>
</tr>
<tr>
<td>Cardioprotective effects</td>
<td>Hydroalcoholic extract</td>
<td>Subcutaneous injections of isoproterenol hemisulphate induced MI</td>
<td>(125)</td>
</tr>
<tr>
<td>Hypolipidaemic activity</td>
<td>Guggulsterone</td>
<td>Triton and cholesterol-fed hyperlipaemic rat models</td>
<td>(126)</td>
</tr>
<tr>
<td>Anticancer activities</td>
<td>Guggulsterone</td>
<td>Inhibits proliferation of a variety of cancer cell lines.</td>
<td>(127, 128)</td>
</tr>
<tr>
<td>Antiobesity effects</td>
<td>Guggulsterone</td>
<td>Adipogenesis effect in 3T3-L1 cells.</td>
<td>(129)</td>
</tr>
<tr>
<td>Antidiabetic activity</td>
<td>Gugulipid</td>
<td>High fat diet induced diabetic rats</td>
<td>(130)</td>
</tr>
<tr>
<td>Neuroprotective effects</td>
<td>Gugulipid, an ethyl acetate extract</td>
<td>Streptozotocin-induced memory deficits in mice.</td>
<td>(131)</td>
</tr>
<tr>
<td>Thyroid function</td>
<td>Gugulu extract</td>
<td>Mice model</td>
<td>(132)</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>Gugulu extract</td>
<td>Systemic review</td>
<td>(133)</td>
</tr>
<tr>
<td>Nodulocystic acne</td>
<td>Gugulu extract</td>
<td>Systemic review</td>
<td>(134)</td>
</tr>
</tbody>
</table>
4.4.4 Oleo-gum-resin of Salai guggul

4.4.4.1 Botanical source

It consists of oleo-gum resin of *Boswellia serrata* belonging to family Burseraceae.

4.4.4.2 Common names

- Beng. – Luban, Salai
- Eng. – Indian Olibanum tree
- Guj. – Saledo, Dhupdo
- Hind. – Luban, Salai
- Kan. – Madi
- Mal. - Kunturukkam
- Mar. – Salayi
- Tam. – Parangisampirani
- Tel. - Sambrani (135)

4.4.4.3 Chemical constituents

**Major:** Volatile oil (9 %), gum and resin; the volatile oil contain α- thujene, α-phellandrene, β- phellandrene, α- terpineol, limonene, camphene, myrcene, α-terpene, p- cymene; active principles boswellic acids from resin viz., α- boswellic acid, β- boswellic acid , 3-O-acetyl-β-boswellic acid, 11 –keto- β-boswellic acid, 3-O-acetyl-11–keto- β-boswellic acid.

**Minor:** A diterpene alcohol serratol and four tetracyclic triterpene acids 3-α-acetoxytirucall-8, 24-dien-21-oic acid, 3-ketotirucall-8, 24-dien-21-oic acid ,3-α-hydroxytirucall-8, 24-dien-oic acid, 3- β-hydroxytirucall-8, 24-dien-21-oic acid. (135)
4.4.4.4 Traditional uses

Ayurveda describe the antirheumatic (antiarthritis) activity of gugguls-the gum-resins of trees (136-140). In addition to its beneficial use for arthritis, this gummy resin is also mentioned in traditional Ayurvedic and Unani texts as an effective remedy for diarrhoea, dysentery, ringworm, boils, fevers (antipyretic), skin and blood diseases, cardiovascular diseases, mouth sores, bad throat, bronchitis, asthma, cough, vaginal discharges, hair-loss, jaundice, hemorrhoids, syphilitic diseases, irregular menses and stimulation of liver. It is also diaphoretic, astringent, diuretic and acts both as internal and external stimulant. Modern medicine and pharmacology strongly point out to its use as an antiarthritic, antiinflammatory, antihyperlipidemic (controls blood lipids), antiatherosclerotic (anticoronary plaque), analgesic (pain-reliever) and hepatoprotective (protects the liver). (141-145)

4.4.4.5 Pharmacological actions reported

*Boswellia serrata* has made important contribution to the field of science from ancient times to modern research due to its large number of medicinal effects. In light of that an brief review presented in Table 4-6
### Table 4-6 Pharmacological actions of *Boswellia serrata*

<table>
<thead>
<tr>
<th>PHARMACOLOGICAL ACTIVITY</th>
<th>EXTRACT USED</th>
<th>EXPERIMENTAL MODEL</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-inflammatory</td>
<td>Methanolic</td>
<td>TNFalpha, IL-1beta, and NO inhibiting <em>in vitro</em> assay</td>
<td>(146)</td>
</tr>
<tr>
<td>Anti-oxidant</td>
<td>Methanolic and aqueous extract</td>
<td>Ferric-Reducing Antioxidant Power (FRAP) Assay</td>
<td>(147)</td>
</tr>
<tr>
<td>Anti-Ulcer</td>
<td>Petroleum ether and Aqueous extracts</td>
<td>Pyloric ligation, ethanol/HCl, acetyl salicylic acid, indomethacin and cold restrained stress-induced ulceration</td>
<td>(148)</td>
</tr>
<tr>
<td>Anti-arthritic</td>
<td>Gum resin exudate</td>
<td>Carrageenan-induced rat hind paw oedema</td>
<td>(149)</td>
</tr>
<tr>
<td>Anti-asthmatic</td>
<td>Ethanolic extracts of the gum resin</td>
<td>Respiratory volumes and serum marker test</td>
<td>(150, 151)</td>
</tr>
<tr>
<td>Anti-atherosclerotic</td>
<td>Aqueous and hydroalcoholic extracts</td>
<td>Lipopolysaccharide (LPS) induced nitric oxide (NO) production by macrophages under in vivo and in vitro conditions</td>
<td>(152)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Acetyl-11-keto-β-boswellic acid (AKBA)</td>
<td>Human prostate tumor xenograft mice</td>
<td>(153)</td>
</tr>
<tr>
<td>Anti-diarrhoeal</td>
<td>Gum resin extract</td>
<td>In vitro experiments with terminal ileum of guinea pig</td>
<td>(154)</td>
</tr>
<tr>
<td>Hepato-protective</td>
<td>Hexane extract of oleo-gum-resin</td>
<td>Liver injury induced by carbon tetrachloride, paracetamol or thioacetamide.</td>
<td>(155)</td>
</tr>
<tr>
<td>Anti-microbial</td>
<td>Acetyl-11-keto-β-boswellic acid</td>
<td>Activity on different pathogenic strain Postantibiotic effect (PAE) and biofilm susceptibility assay</td>
<td>(156-158)</td>
</tr>
<tr>
<td>Wound healing</td>
<td>Oleo gum resin fraction</td>
<td>Excision wound model</td>
<td>(159)</td>
</tr>
<tr>
<td>Diuretic</td>
<td>Aqueous</td>
<td>Normal albino rat</td>
<td>(160)</td>
</tr>
<tr>
<td>Analgesic</td>
<td>Non-phenolic fraction</td>
<td>Acetic acid induced abdominal constriction and tail immersion methods</td>
<td>(161)</td>
</tr>
<tr>
<td>Anti-hyperglycemic</td>
<td>Oleo gum resin fraction</td>
<td>Multiple low-dose streptozotocin (MLD-STZ) diabetes</td>
<td>(147, 162)</td>
</tr>
</tbody>
</table>