CHAPTER 2

REVIEW OF LITERATURE
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1. **History of Gold in Medicine**

The history of gold in medicine is quite old. It has been recently reviewed by Kean and coworkers (1997); a gist is given below:

- **2500 BC** Medicinal use known to Chinese.
- **600 BC** Taoist philosophers used it to enhance the quality of medicines.
- **First century AD** Records available regarding its use in medicine by the Roman physician Pliny and Greek philosopher Dioscorides.
- **Up to twelfth century AD** Mention of its extensive use in Arabic and Persian medical literature. Highly valued by pharmacist/physicians of that era including Yabir, Avicenna and Rhazes.
- **1214 – 1292 AD** Knowledge of structural and sophisticated medical practice of gold therapy now spreads to Europe. The French scholar Roger Bacon was one of the first known gold alchemist of the British Isles.
Up to eighteenth century

Considerable literature available on all chemical and medicinal aspects of gold. Physicians attach great value to this precious metal for e.g. Paracelsus proclaimed gold compounds as panacea but many of his contemporaries refuted his theories.

Nineteenth century

Modern use of gold compounds in medicine was initiated in early part of the century by two professors at University of Montpellier: Andre-Jean Chrestien and Pierre Figuier. Uses mentioned in infectious diseases including tuberculosis and syphilis. In that period rheumatoid disease was erroneously considered as an infectious disease but this assumption led to the discovery of its use in rheumatoid arthritis. The initial experiments were amazingly successful and over the next 50 years a lot of effort revealed its beneficial and toxic effects. A number of therapeutically useful gold compounds were introduced. Myocrysin and gold sodium thiomalate were the most frequently used preparations but these could be given by parenteral route only. In the late 1970's auranofin emerged as a possible alternative gold anti-arthritic agent with advantage of being orally effective.
2. Distribution in Eco-System

It is widely distributed in the environment at low concentration. The average content in earth's crust is 5 μg/kg and in sea water 3-4 mg/ton. The values for content and acceptable guidelines in drinking water are not available. Cyanogenetic plants are known to concentrate this metal up to 180 μg/g. Animal food contain 0.03 – 1 mg % gold. (Merian 1991)

3. Distribution in Human Body

The average content in adult man (70 kg) is < 10 mg (Schroeder 1976). It is widely distributed; data is available for at least 30 human tissues and body fluids including adrenal, aorta, bones, brain, diaphragm, gastrointestinal tract (caecum, colon, duodenum, ileum, jejunum, rectum, stomach), heart, kidney, larynx, liver, lung, muscle, nails, oesophagus, omentum, ovary, pancreas, prostate skin, spleen, testis, thyroid, tooth, trachea, urinary bladder and uterus. The content in most of tissues is < 10 ppm. Bones contain half of the body burden (Schroeder 1976). The values for blood are quite high (μg/l): total (0.37), erythrocytes (0.80), plasma (0.06) and serum (0.08). (Iyenger et al 1978; Vohora 1982; Athar and Vohora 1995)

4. Production and Industrial Uses

The world production of gold is estimated to exceed 1 million tons. The major gold producing countries are Canada, Soviet Union, South Africa, China and USA. The metal is mainly extracted by gravitational processes, amalgamation and cyanide extraction. Beside principle usage in jewelry the metal is attributed with other industrial (electronic
industry, catalytic processes, gold electrodes for analysis of metals such as arsenic, copper, chromium and mercury) and medicinal usage (dentistry, neuronal imaging with colloidal gold, chrysotherapy in rheumatoid arthritis). It is not a major environmental hazard. The amount of gold released to the environment, however, is not appreciable. It depends on several factors including its chemical nature (inert, melting point and boiling point), recovery and recycling. The latter are obviously efficient because of its preciousness which acts as an economic incentive. There are no known incidents of environmentally-induced gold toxicity. (Pol 1989; Merian 1991; Athar and Vohora 1995)

5. Absorption, Distribution, Metabolism and Excretion

5.1 Absorption

The main source of gold to humans are gold containing drugs. Auranofin is rapidly but incompletely absorbed after oral administration. Auranofin contains aurous gold. It is stabilized by sulphur and phosphorus ligand indicating hydrophobic nature of gold without ionic charge. These facilitate absorption of compound following oral administration (Lorber et al 1982). The serum of healthy subjects (not treated with gold-containing compounds) contains <0.05 ng/ml of gold (Gottlieb 1982). High concentration of gold (90 and 70 μg/g) were found in two skin biopsy samples taken from under gold rings (Brown et al 1982). The use of intravenous and oral ¹⁹⁵Au labeled auranofin in dogs has suggested that approx. 25% of an oral dose is absorbed (Chaffman et al 1984).

The amount of gold present in plasma or serum have been virtually identical during auranofin treatment. The mean peak plasma concentration of 0.066-0.23 μg Au/ml following single dose of 6 mg/kg whereas 0.085 μg Au/ml after 6 mg dose were
administered to patients who had been treated auranofin 3 mg twice daily for 6 months. The steady state plasma gold concentrations are dose-dependent and range from 20-100 μg/ml with auranofin 2-9 mg daily for 8-12 weeks (Chaffman et al 1984). Furthermore gold is 95% bio-available when administered intramuscularly (Blocka et al 1986). The absorption of hydrogen cyanide from tobacco smoke alters the distribution of gold compounds *in vivo*. A substantial fraction of the gold in the mammalian cell cytosol is metallothionein bound. (Merian 1991)

5.2 Distribution

5.2.1 Tissue distribution

The distribution of gold in different tissues estimated are less than value of $10^{-8}$ to $10^{-4}$ g (Synder et al 1975). Liver, skin and muscle may have higher contents than adipose tissue. Gold is carried to plasma to all parts of the body. More than 75% of injected dose is ultimately found in liver, kidney, skin, hair and other tissues (Stokinger 1981). An autopsy on a patient, 8 weeks after the end of 4 years therapy with 2350 mg gold (as gold thioglucose) revealed higher concentration of gold in organs (lymph nodes, liver, bone marrow and spleen) and lower concentration in articular and pararticular structures, synovium, bone cartilage and muscle. (Stokinger 1981)

Hair and nail have low affinity for gold whether administered orally or by injection (Gottlieb 1982). The deposits of gold compounds are also found in macrophages of many tissues. (Insel 1996).
5.2.2 Plasma protein binding

Binding to plasma protein is estimated to be 59.9% of the mean whole blood concentration of auranofin gold (1.25 µg/ml) in patients who had undergone at least three months of auranofin treatment (Herrlinger et al 1982). After oral administration of auranofin 25% of the administered dose is detected in plasma-bound albumin and peak concentration of 6-9 µg% are reached with in 1-2 hours (Kean et al 1997).

5.2.3 Synovial fluid

Synovial fluid concentration in a patient treated with auranofin (varying doses) for 18 months was found to be < 0.44 µg/ml (Gottlieb 1982).

5.2.4 Blood

Whole blood contained 0.55 µg/ml following 18 months treatment with auranofin (Gottlieb 1982). About 40 - 42% of whole blood auranofin gold content was associated with circulating cellular elements. The whole blood gold concentration of 2.5 -10 µg/ml, auranofin gold was 23-38% bound to erythrocytes in vitro. Gold aurothiomalate and gold keratinate (5 µg gold/ml) were not bound to erythrocytes (Herrlinger et al 1982). Gold apparently accumulated in the white cells because the cell bound concentration relative to unit volume was up to 20 times higher than plasma levels (Herrlinger et al 1984). When Au colloid were injected intraperitoneally, highest concentration were measured in lymph nodes on day 7 (Williams and Bradley 1989).

5.2.5 Adipose tissue

The total content of gold in adipose tissue of 70 kg reference man estimated 0.011µg/g (Synder et al 1975).
5.3 Metabolism

The reactions of gold compounds with tissues and body fluids depend on oxidation state, the ligand initially bound to gold and environmental accumulated ligands. In the blood, the gold (I) thiolates react with proteins, via ligand exchange reactions. These reactions of gold are generally very labile, and the carrier ligands are displaced, then metabolized in vivo and excreted. The ingestion of 6 mg dose of Au\textsuperscript{195} labeled auranofin revealed that total body retention accounted for 15\% (Chaffman et al 1984). Auranofin, either single dose (17, 34 or 68 mg/kg, po) or multiple doses (0.2, 0.6, 2, 9 and 40 mg/kg/day for 3 – 14 days) showed effect on hepatic and renal drug metabolism. Twenty hours following single dose of auranofin, no effect on hepatic P450, ethoxycoumarin-o-demethylase (ECOD) and benzphetamine-N-demethylase (BPND) were observed. However auranofin at 2 mg/kg reduced hepatic P450 and ECOD activity at 3 days and this effect was reversed with continued treatment for 14 days. The overall result indicate that auranofin administration at doses 20 times the human dose, produced reversible decrease in hepatic and renal P450 which may be result of altered haem metabolism (Leonard et al 1986).

The metal primarily concentrates in liver and kidney, then gets slowly mobilized to other tissues and becomes more widely dispersed. The half-life of gold transport to deep storage compartment is quite long (Merian 1991). After gold injection the contents of gold, copper and zinc in kidneys and liver were found to increase. Mammalian cell cytosol contains metallothionein-bound fraction of gold (Saito et al 1997).

5.4 Excretion

The ingestion of 6 mg dose of \textsuperscript{195}Au labeled auranofin revealed cumulative fecal and urinary excretion for 79.7\% (Chaffman et al 1984). On the basis of human and rat studies,
the high fecal gold content associated with auranofin is principally due to incomplete absorption. Secretion of gold through the intestinal wall and via the biliary tract are probably contributory (Blocka et al 1981). Long term treatment with auranofin (3 mg twice daily for 21 months) resulted in steady state rate of urinary gold excretion of 250-400 µg/day (Chaffman et al 1984). The normal values for gold in urine are ≤ 20 µg/l. The excretion of gold is 60% to 90% renal and 10-40% fecal after a cumulative dose of 1g of gold. Sulfahydryl agents such as dimercaprol, penicillamine and n-acetyl-cysteine increase the excretion of gold. After cessation of the treatment, half-life of the gold in the body is about 80 days (Insel 1996).

6. Biologically Active Gold Compounds

These are summarized in Table 2.1 (Lewis and Walz 1982 and Insel 1996)

Table 2.1: BIOLOGICALLY ACTIVE GOLD COMPOUNDS

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Trade Names</th>
<th>% Au</th>
<th>Formula</th>
</tr>
</thead>
</table>
| Gold sodium thiomalate (GST),         | Myochrysin,               | 50.5 | \[CO_2Na \]
| Disodium aurothiomalate              | Myocrisin,                |      | AuSCH_2CO_2Na             |
|                                       | Tauredon                  |      |                          |
| Gold thioglucose,                     | Solganal                  | 50.3 | \[\underline{\text{CH}_2\text{OH}} \]
<p>| Aurothioglucose                      |                           |      | \underline{\text{O}} \text{SAu} |
|                                       |                           |      | [\underline{\text{HO}} \underline{\text{O}} ] |
|                                       |                           |      | PhNHCOC\text{H}_2\text{SAu} |
| Gold thioglycoanilid,                 | Lauron                    | 54.2 |                          |
| Aurothioglycolanilid                 |                           |      |                          |</p>
<table>
<thead>
<tr>
<th>Substance</th>
<th>Name(s)</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold sodium thiosulphate</td>
<td>Sanochrysine, Sanocrisin, Crisalbine, Solfocrisol, Thiochrysine</td>
<td>Na$_3$Au(S$_2$O$_3$)$_2$</td>
</tr>
<tr>
<td>Aurothiosulphate sodium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium aurothioglycolate</td>
<td>Myoral</td>
<td>(AuSCH$_2$CO$_2$)$_2$Ca</td>
</tr>
<tr>
<td>Sodium 2-aurothiobenzimidazole-4-carboxylate, aurothiol</td>
<td>Triphal</td>
<td></td>
</tr>
<tr>
<td>Gold sodium 3-thio-2-propanol-1-sulphonate, sodium 3-aurothio-2-hydroxypropane-sulphonate</td>
<td>Allochrysine</td>
<td></td>
</tr>
<tr>
<td>Sodium auroallylthiourea-m-benzoate</td>
<td>Lopion</td>
<td></td>
</tr>
<tr>
<td>S-Triethylphosphine gold 2,3,4,6-tetra-O-acetyl-1 thio-β-D-glycopyranoside</td>
<td>Auranofin (AF) (SK&amp;F D-39162), Ridaura</td>
<td></td>
</tr>
<tr>
<td>Chloro(triethylphosphine) gold</td>
<td>SK&amp;F 36914</td>
<td>Et$_3$PAuCl</td>
</tr>
</tbody>
</table>
7. Oral vs Parenteral Gold Compounds

The parenterally administered gold compounds have been used for decades for the treatment of rheumatoid arthritis. The new gold compound i.e. auranofin can be partially absorbed in the gut following oral administration due to its highly lipophilic nature (Juan 1984) which is probably the main cause for difference in kinetic properties vs the parenteral gold compounds. Auranofin is 20-25% orally absorbed and has less body retention, greater fecal excretion and urinary excretion than injectable gold sodium thiomalate (Furst 1983). Auranofin (3 mg twice a day) is superior to placebo therapy, with similar efficacy and greater safety than that with injectable/parenteral gold compounds. (Blodgett 1983) The mechanism of action of auranofin is probably similar to parenteral gold compounds. The auranofin affects polymorphonuclear cells and monocytes at lower concentration that gold sodium thiomalate and generally affects humoral and cell mediated immunity in the same direction (Furst 1983). Auranofin does not influence g.i.t side effects by increasing the dose (Johannsen et al 1997)
Chapter 2.2

HEALTH EFFECTS

1. Physiological Role
At the present state of knowledge gold is classified as non-essential trace element with no known biological functions. The biological classification of element is, however, fluid and is constantly changing with advancement of knowledge (Vohora and Dobrowolski 1990).

2. Systemic effects
2.1 Gastro-intestinal system
Auranofin appears to be better tolerated than injectable gold compounds (Insel 1996). It is, however, associated with high incidence of gastro-intestinal disturbances such as colitis, loose stools, probably caused by inhibition of Na⁺K⁺ATPase (Bross et al 1987). Diarrhea and abdominal cramps that are often dose-related and resolve spontaneously (Tozman and Gottlieb 1987). Gold-induced enterocolitis has also been reported with parenteral gold (Chaffman et al 1984).

2.2 Cardiovascular system
Anemia has been reported to occur occasionally either as direct or secondary effect to haematuria, leucopenia, lymphopenia and neutropenia observed in 13% of patients. Thrombocytopenia, though rare (<0.5% of patients), has been reported following auranofin administration (Chaffman et al 1984). Gold containing compounds are potent
and selective inhibitors of endothelium-dependent relaxation. Among all gold compounds, auranofin is most potent in producing approx. 40% relaxation at 1 μmole concentration (Ohlstein and Horohonich 1989). Auranofin reduces the acetylcholine induced relaxation in the isolated rat aorta. The contraction induced by phenyephrine and 5-HT are enhanced by auranofin (Fontaine et al 1991).

2.3 Respiratory system

Gold induced lung disease (pulmonary disease) mostly appears in the course of gold therapy. It usually improves with the cessation of therapy or treatment with corticosteroids (Tomioka and King 1997). Gold salts have been shown to reduce the need of systemic corticosteroid treatment for asthma. Auranofin provide an effective adjunct to treatment for steroid dependent asthma, leading to reduction of oral steroid dose (Nierop et al 1992).

2.4 Nervous system

The colloidal gold is useful as a tracer for physiological studies for neuronal transport in vivo and in vitro (Pol 1989). Gold thioglucose can be used to induce experimental obesity possibly mediated through damage to the hypothalamus (Merian 1991).

2.5 Skin

Skin reactions to gold jewelry are rare and occur most frequently among chrysotherapy patients (Raspon 1984). Recently gold sodium thiosulphate was found to be the most common sensitizer after nickel sulphate in routine patch tested dermatitis patient (Bruze et al 1995).

Radiation keratosis occurred in woman who wore radioactive gold ring (Helm et al 1996). Stomatitis occur concomitantly with skin rash in 1-12% of patients. The
development of mouth ulcer is definite contraindication to gold therapy. Oral ulceration can precede the development of pemphigoid like bullous skin lesions (Kean et al 1997).

2.6 Renal system
Proteinurea and haematuria occurs in up to 5% of patients treated with gold compounds. The gold compounds administration in the form of soluble organic or inorganic salts caused tubular damage in experimental animals (Athar and Vohora 1995; Kean et al 1997).

2.7 Eye
Excess gold, may elicit toxic effects on eye following continued therapeutic use because the metal is known to accumulate in the human eye. Conjunctivitis has been reported from collective studies in 10% of patients treated with gold compounds (Grubb and Bentley 1988; Kean et al 1997).

2.8 Infections
Inhibition of Mycobacterium tuberculosis in vitro lead to its use in tuberculosis and other infectious disease. Gold therapy in 35 tuberculous cases resistant to streptomycin, isoniazid, paraaminosalicylic acid resulted in gradual improvement of general conditions (71.35% cases) without any radiological deterioration (Koch 1980; Vohora and Dobrowolski 1990). Gold compounds are also useful in the treatment of HIV associated psoriatic arthritis (Shapiro and Masci 1996). Colloidal gold is used as a marker for detection of rota-virus in feces. This virus is the main cause of severe diarrhoea in infants and children (Fernadez et al 1994).
2.9 Other effects

Radio colloidal gold is considered superior to all kind of radio-therapy; it also controls the contraletal pleural dissemination (Sattler 1970). Implantation of gold weights in to the upper eyelid for rehabilitation of eye lid closure showed satisfactory results. These implantation procedures are also preferred for treating patients with complete paralysis. Gold implants caused reanimation of paralyzed face (Moser and Oberascher 1997). Gold compounds have also been shown to exert an effect on copper and zinc homeostasis (Chaffman et al 1984). Gold thioglucose is reported to elicit anti ovulatory effect in rats and mice through the inhibition of CNS pituitary mechanism (Athar and Vohora 1995).
Chapter 2.3

EFFECTS ON IMMUNE FUNCTION

1. Concept of Immunomodulation in Indian Systems of Medicine

The modulation of immune response by using natural products for therapy has become a subject of scientific investigation all over the world. The basic concept always existed in the ancient systems of medicine: Ayurveda and Unani-Tibb which follow a host-oriented approach. One of the fundamental therapeutic strategies in these systems is to provide protection or treatment by strengthening body’s resistance to infections. It is now being increasingly recognized that it is possible to modulate host’s immune system to enable it to cope with the infection challenge and environmental pollution. Immunomodulation may involve immunostimulation or immunosupression. The former is desired for the treatment of non-specific immune deficiency while the latter is useful in cases of allergy, autoimmunity or transplantation.

2. Immunomodulation by Metals

A large number of metals are known to modulate immune response. The alteration of immune function by metals has also been demonstrated at the cellular level. Both lead and nickel enhanced the plaque forming cell response to T dependent antigen, sheep rat blood cells while calcium, chromium, copper and zinc inhibited these parameters (Smith and Lawrence 1988). It is a well known that chronic uremia is associated with depression of immune system. Zinc supplementation (120 mg ZnSO₄) failed to restore the immune
parameters but enhanced antibody response to multivalent influenza vaccine (MIV) in haemodialysis patients (Turk et al 1998).

3. Immunomodulation by Gold

Gold therapy is most ancient. Its recent incarnation is in the form of delivery vehicle for gene therapy. Its administration to humans is both deliberate and inadvertent. It is universally recognized as the most inert of the metals, yet it can be sensitizing. Gold’s widespread clinical application (in rheumatoid arthritis) was derived from a premise that was totally flawed (See Chapter 2.1 and Section 1).

Gold compounds suppress immune responsiveness. Clinical observations in patients treated with gold compounds show that immunoglobulins levels and rheumatoid factor titers often decrease (Lorber et al 1978; Hashimoto et al 1992). The gold salts could play an important role in disease modification of rheumatoid arthritis by chrysotherapy through inhibition of synovial lymphocyte proliferation as it significantly inhibits the proliferative responses of cultured T cells stimulated by interleukin –2 (IL-2) (Wolf and Hall 1988). Though it is employed clinically for immunosupression yet it can endanger toxicities from immunostimulation. Gold compounds are regulators of immunological response and have been exploited for their beneficial effects. A number of hypotheses were postulated regarding the mechanism of action of gold in inflammatory processes particularly those involving protein and enzyme interactions. Major sites of biological action of gold compounds are shown in Figure 2.1.
POSTULATED SITES OF BIOLOGICAL ACTIONS OF GOLD

PROTEINS

MICROBES

METALS

ENZYMES

CELLS

Regulatory
- Macrophages
- Helper or inducer T cell
- Suppressor T cell (T3)

Effector
- Mast cell
- Platelets
- B cells
- Effector T cells
- Cytotoxic T cell
- Killer cells
- Polyphonuclear leucocytes

Figure 2.1 Source: Lewis and Walz (1982)
3.1 *Gold and Autoimmune Disorders*

Metal induced autoimmunity is well established but poorly understood phenomenon. Heavy metals like cadmium, gold and mercury have strong association with autoimmunity. Treatment of rats and mice with cadmium results in autoimmune responses that vary with species and strains of animals. The autoimmune effects of gold preparations are well documented. Gold may cause auto-immune thrombocytopenia, immune complex-mediated glomerulonephritis and other auto-immune disorders. Similarly mercury can induce auto-immune diseases both in experimental animals and humans (Bigazzi 1994).

Rheumatoid arthritis (RA) is a common autoimmune disorder, most often affecting women from 40 - 60 years old. The major symptoms are chronic inflammation of joints, although the hematopoietic, cardiovascular and respiratory system are also affected. Chrysotherapy (Greek *Chryos*: gold) is the use of gold compounds in this disease. The first successful use of chrysotherapy in this disease was reported by Forestier in 1929 (Lewis and Walz 1982). Gold compounds, cause suppression of autoimmune antibody production and inflammation in genetically autoimmune-prone mice (Friedman et al 1990). While the precise mechanism of action in rheumatoid arthritis is not clear, gold compounds may suppress immune responsiveness (Lorber et al 1978).

3.2 *Gold and Cell-mediated Immunity*

The cell mediated immune reactions participate in the pathogenesis of rheumatoid arthritis. Gold has been shown to influence these responses *in vitro* and *in vivo* with considerable variation in the nature of response. Gold compounds, auranofin and sodium aurothiomalate have been shown to affect cell mediated immunity. This is evidenced by
their influence on oxazolone-induced contact sensitivity and delayed hypersensitivity to sheep red blood cells (SRBC) in mice (Walz et al 1981). Auranofin (1.25, 5 and 10 mg gold/kg) was capable of stimulating compromised oxazolone-induced contact sensitivity. Auranofin (5 mg gold/kg) stimulated immune response in mice which had been immunosuppressed with methotrexate (Walz et al 1982a). Auranofin (0.2 μg gold/ml) showed direct inhibitory effect on polymorphonuclear cells but it elicited no effect on antibody or target cell components of the reaction. The antibody-dependent cellular cytotoxicity (ADCC), mediated by rat peripheral blood polymorphonuclear leukocytes, was markedly decreased (> 94%) by auranofin (Di Martino and Walz 1980). Effects of auranofin on lymphocyte ADCC in vitro appear to be concentration-dependent (Russell et al 1982). Depressed baseline ADCC in vivo in patients with rheumatoid arthritis was corrected by auranofin (6 mg daily) administration (Finkelstein et al 1982).

3.3 Gold and Humoral Immunity

Auranofin (10 mg gold/kg/day) suppressed haemagglutinin antibody response to SRBC (Walz et al, 1982) in adjuvant arthritic rats but not in sensitized mice. Auranofin (2.5, 5 and 10 mg gold/kg) stimulated delayed type hypersensitivity (DTH) of mice to SRBC, without altering their humoral response (Walz and Griswold 1978). Both auranofin and gold sodium thiomalate have been reported to cause the reduction in immunoglobulins levels (IgG, IgM and IgA) and rheumatoid factor during chrysotherapy. The administration of gold sodium thiomalate impaired mononuclear cell function and showed significant reduction in total lymphocytic number (both T and B lymphocytes) (Hassan et al 1986).
Peak reduction in immunoglobulins sometimes correlates with maximum clinical benefits after gold administration. It is unlikely that this decrease in serum antibodies is a result of non-specific inhibition of protein synthesis. All of the serum proteins are not reduced; albumin may increase after auranofin and GST therapy. Gold at high concentration (> 3μg/ml) is known to bind to IgG, IgM and IgA as well as to immune complexes and influence antibody production in vitro. The binding of gold compounds to immune complexes, too, has been suggested to play an important role in delivery and accumulation of gold in reticuloendothelial cells (Lewis and Walz 1982).

3.4 Gold and Non-specific Immunity (Phagocytosis)

Gold compounds (auranofin and gold sodium aurothiomalate) are reported to cause significant suppression of phagocytosis of Candida albicans by human polymorphonuclear cells. (Davis et al 1982). The oxidative metabolites produced during activation of polymorphonuclear cells by particulate matter are collectively termed as "respiratory burst" and can be measured by chemiluminescence assay. Auranofin (0.5–2.5 μg/ml gold) significantly decreased chemiluminescence in zymosan-stimulated human PMN leucocytes (Davis et al 1982) and decreased superoxide production during immune complex and "frustrated" (where immune complexes were fixed to micropore filters) phagocytosis (Roisman et al 1982). It is possible that gold compounds may exhibit a differential effect on the phagocytic capacity of cells. Gold sodium thiomalate (100 μg/ml) reduced the in vitro phagocytic capacity of peripheral human blood monocytes towards Candida but did not influence the phagocytosis of cultured macrophages. After treatment with gold, the digestive capacity was normal in the peripheral blood monocytes but suppressed in macrophages (Lewis and Walz 1982).
Gold also seems to inhibit the differentiation of the drug exposed blood monocytes to macrophage. Inhibition of phagocytosis after gold treatment *in vivo* is at its peak when the level of non-albumin bound gold (free reactive gold) is maximum (Lewis and Walz 1982). This suggests that the reactive form of gold may be binding to the cell surface and consequently interfering with membrane interaction between cells and the phagocytosable material.
Chapter 2.4

MEDICINAL USES AND TOXICITY

1. Medicinal Uses

1.1 Rheumatoid Arthritis

Principal medical indication of this metal is in rheumatoid arthritis. Gold compounds have been employed as therapeutic agents for rheumatoid arthritis since 1929. The most commonly used drugs are auranofin, gold sodium thiomalate and gold sodium thioglucose (Huskiesson 1976). The relapse rate with gold therapy for treatment of rheumatoid arthritis are usually very high. Out of every 100 patients under treatment with gold, only 20 will show recovery and 25-40 will develop toxic reactions; half of them sufficient serious to require discontinuation of therapy (Athar and Vohora 1995). Its use is usually reserved for patients with progressive disease who do not get relief from NSAID’s therapy (Edmonds et al 1993). Gold compounds often arrest the progression of the disease involved joints, prevent involvement of unaffected joints, improve grip strength and morning stiffness. The therapy with gold is also beneficial in juvenile arthritis, palindromic rheumatism, psoriatic arthritis (Insel 1996). The combined treatment with oral and injectable gold provides a new approach for chrysotherapy with an increased anti-arthritis potency. It is evidenced by one clinical study, when auranofin, given in combination with gold sodium thiomalate (GST) showed increased anti-arthritis capacity vs those treated with individual gold compounds (Finkelstein et al 1988).
Auranofin when added to regimen of salicylates and/or a non-steroidal anti-inflammatory drug for the treatment of rheumatoid arthritis, significant additional therapeutic benefit was observed (Blodgett et al 1984).

1.2 Pruritis

Gold, in elemental form, has been employed for centuries as an anti-pruritic agent to relieve itching palm (Insel 1996).

1.3 Dental

The gold compounds (amalgams of gold) are used as dental material for the restoration of teeth and dental fillings. (Giblin 1989; Merian 1991)

1.4 Pemphigus

Gold compounds (gold sodium thiomalate) caused a reduction in serum pemphigus antibody titres. Hence it may be used as treatment of choice for the management of pemphigus. The exact mechanism is not known but the effects may be attributed to reduction in anti-epithelial antibody titre (Lewis and Walz 1982).

1.5 Asthma

A number of reports published in 1930's suggested that chrysotherapy exerts a beneficial effect in asthma. Treatment with gold aurothiomalate showed improvement in patients with extrinsic asthma vs the placebo treated subjects. Long-term treatment with gold compounds reduce the bronchial responsiveness to acetylcholine (Lewis and Walz 1982). Prolonged administration of oral corticosteroids in patients with asthma may be associated with serious side effects. Auranofin provides an adjunct to the steroid-dependent asthma and helps in reduction of the steroid dose (Nierop et al 1992).
1.6 **Cancer**

Radioactive gold ($^{198}$Au) has been used, in the form of colloidal solution, for radiotherapy in cancer of GIT, skin, connective tissue, bladder, pleura, ovaries, prostate and pituitary glands (Athar and Vohora 1995). The antioxidant efficacy of gold compounds (auranofin and gold sodium thiomalate) was examined by studying their effects on the generation of reactive oxygen species (ROS). Auranofin showed inhibitory effect on ROS production and OH$^-$ generation was found to be significantly depressed in a dose-dependent fashion; GST produced slight inhibition at higher concentrations. These findings suggest that auranofin may play an important role in inhibition of respiratory burst and generation of inflammatory reactions (Miyachi et al 1987).

Auranofin, when complexed with active anti-tumour agents and non-active agents, demonstrated significant anti-tumour effects against P388 leukemia in mice. Auranofin has also been shown to inhibit DNA synthesis and to a lesser extent RNA and protein synthesis. The ability of gold compounds to inhibit metabolic enzymes should also be considered as a possible mechanism of action in prevention of tumour growth. Thus auranofin or related gold analogues promise an exciting new therapeutic approach to cancer chemotherapy (Lewis and Walz 1982).

1.7 **Other Uses**

Gold compounds were earlier used for various infectious diseases like leprosy, syphilis, tuberculosis without any remarkable success. Such usage is now of historical interest only as effective and safe drugs are available for the purpose. Gold (WGA-apo HRP-Au) compound is used as retrograde tracer to study or describe the localisation, organization and density of lateral hypothalamic neurons projecting to central amygdaloid nucleus.
Colloidal gold serves as an excellent immunomarker at the ultrastructural level. It is absorbed to immunoglobulins hence can be used for the light microscopic observations. The different size gold particles, absorbed to different antibodies can be used for pre and post embedding staining of different antigens in same tissue (Pol 1989). Beauty parlours in major metropolitan cities advertise remarkable cosmetics effects of gold facial. Scientific reports on this aspect are not available.

2. Toxicity

2.1 Acute Toxicity

Gold toxicity is uncommon and drug-related deaths are rare. The acute toxicity of auranofin evaluated in Charworth Swiss mice and Charles river rats, in which median lethal oral dose were 310 and 265 mg/kg respectively (Saunders 1983). The acute toxicity for intramuscular aurothiosulphate was tested and LD$_{50}$ was found to be 35 mg/kg in rats. The intramuscular injection of gold sodium thiomalate and gold thioglucose are painful and produce high initial gold concentration in serum. Such high levels may damage the kidney (Stokinger 1981).

2.2 Chronic Toxicity

Auranofin (1, 3.5 and 10 mg/kg daily for 1 year) produced dose-related changes including weight loss, excess salivation, soft feces, anaemia, leucocytosis (Chaffman et al 1984). The haematological abnormalities were believed to secondary to auranofin induced gastrointestinal lesions, rather than direct effect on bone marrow (Payne and Arena 1978). Oral (auranofin) and injectable (gold thiomalate) gold compounds, when administered for 6 months, showed respectively 45-58% and 4% of gold present in blood
cell (Sharma et al 1985). Gold-induced peripheral neuropathy is also seen after aurothioglucose (255 mg) administration (Weiss et al 1982).

Gold compounds may cause chronic contact dermatosis. Skin rashes occur at doses >250 mg gold compounds (Stokinger 1981). The growing incidence of hypersensitivity induced by gold compounds has moved gold compounds in the second place of allergen following nickel (Hostynek 1997). Development of a “gold nephrosis” syndrome (edema of face and ankles) has been reported in patients treated with gold compounds. Auranofin inhibits DNA, RNA and protein synthesis at cytotoxic concentration but showed no selective effects. The cytotoxicity and cellular association of gold were found to be dose, time and temperature dependent (Mirabelli et al 1985).

Gold compounds may cause a variety of other adverse reactions including agranulocytosis, alopecia, anaemia, arteritis, corneal chrysis, opacities, conjunctival discolouration, diplopia, encephalitis, enteritis, erythma, fibrosing alveolitis, glossitis, hypokalaemia, lichenoid eruptions, nystagamus, ocular pulsis, pulmonary infiltration, polyarteritis nodosa, purpura, toxic epidermal necrolysis and vasomotor crisis (Davies 1985).

### 2.3 Reprotoxicity

The fetal abnormalities associated with gold therapy were abdominal defects such as gastroschisis and hernia. Abnormalities of brain, heart, lung and skeleton were seen less frequently (Szabo et al 1978). All gold compounds can cause adverse effects during pregnancy related to prolongation of gestation and labour period. Constriction of ductus arteriosus, persistent fetal circulation, impairment of renal function and bleeding are risks during third trimester (Ostensen and Ramsey 1998). Edema was the only major defect
noted in fetal offspring from pregnant rat administered auranofin (0.1-23.2 mg gold/kg/day) during days 6-15 of pregnancy. Auranofin induced abortion, increase resorption, decreased litter size and fetal weight when administered at a dose of 0.1 – 22.6 mg/kg/day to pregnant rabbits (Chaffman et al 1984).

2.4 Immunotoxicity by Gold

The adverse immune reactions develop in up to 30% of patients treated with gold compounds. Gold compounds are reported to cause lack of skin reactivity. The oxidation of Au (I) to Au (III) appears to be responsible for adverse immune reactions which may develop during the gold therapy (Goebel et al 1995).

2.5 Contraindications

2.5.1 Pregnancy and lactation

Gold is contraindicated during pregnancy and breast feeding (Kean et al 1997).

2.5.2 Pathological conditions

- Gold should not be given to patients with renal disease, hepatic dysfunction or hepatitis or hematological disorders (Insel 1996).
- Auranofin should not be administered after the occurrence of several additional gold induced disorders including pulmonary fibrosis, necrotising enterocolitis and exfoliative dermatitis (Insel 1996).

2.5.3 Drugs

- Concomittant use of anti-malarial, immunosupresssants, phenylbutazone or oxyphenbutazone is contraindicated because these drugs might cause blood dyscriasis (Insel 1996).

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2.6 Mechanism of Toxicity
- Gold resistance i.e. exposure to metals induce cell adaptation and resistance in both prokaryotic and eukaryotic cell lines (Wollheim 1988).
- Partial or complete myelosuppression
- Renal toxicity: depletion of large amount of gold in lysosomes in renal epithelium (Ghadially et al 1982).

2.7 Antidote, Therapeutic Measures
- Chelation with BAL. (Athar and Vohora 1995)
- Steroids for pneumonitis and supportive care.
- 2,3 dimercaptosuccinic acid was the most effective antidote for gold (sodium bis [thiosulphato] gold [I]) intoxication in mice (Basinger et al 1985).

2.8 Carcinogenicity
Injection of gold thioglucose (400 mg/kg) to female mice showed an increase in incidence of memory cancer (38/38 treated vs 29/34 untreated) and reduced average time of 50% tumour appearance (240 days vs 315 days in controls) (Stokinger 1981).
Chapter 2.5

GOLD PREPARATIONS USED IN INDIAN MEDICINE

1. Calcined Metal Preparations: Philosophy and Present Status

1.1 Concept of Calcination

Indian systems of medicine use a number of mineral preparations. These are mostly used in calcined forms: as *Bhasmas* in Ayurveda, *Kushtas* in Unani-Tibb and *Parpams* in Siddha system of medicine. Calcination involves ashing of the metal using specialized traditional techniques. *Bhasma* means burning, *Kushta* means to kill; it is believed that the metal is killed by burning. The techniques involves incorporation of medicinal plants/herbal juices during the ashing process. Unani physicians state that the ‘soul’ of the plant is incorporated into the body of the metal. This changes the properties of the toxic metal making it therapeutically useful and safe (Habeeb 1987).

1.2 Advantages of Calcined Mineral Preparations

The claimed advantages of calcined mineral preparations vs other drugs can be summarized as follows:

a) Effective in minute quantities

b) Rapid action because of deep penetration

c) No toxic / side effects

d) Long shelf life

e) Usefulness in obstinate / incurable diseases

f) Usefulness in patients with weak constitution
g) General tonic rejuvenating and *Rasayana* properties

h) Compact dosage forms make easy to carry during journey

i) No interaction with plant-based drugs

j) Palatable and so better acceptance by patients

1.3 Present Status

Unfortunately these claims have not been subjected to scientific investigations. While the Government of India is spending considerable amount of funds in research on medicinal plants, there has been practically no effort in research on metallic preparations. As a result the survey of literature (mostly from western countries) shows only the toxic and adverse effects of these metals. This gives biased and one sided picture with gradual loss of faith in drugs of mineral origin.

1.4 The Questions

There is an urgent need to look into several questions in this respect:

a) Are these agents effective and safe?

b) Do the herbal juices change the properties of the metals and detoxify them as claimed? If so, How?

c) What are the chemical forms of the metals present in these preparations?

d) What is their exact chemical composition?

e) What are their pharmacokinetic properties? Information on their absorption, distribution in different tissues and body fluids, metabolism and excretion?

f) Are these preparations standardized or variations exist in different batches or products from different manufacturers?

g) What are their systemic effects?
h) What are their LD₅₀/MTD/Therapeutic index values?

i) What is their bio-availability?

j) What experimental and clinical data is available on these agents?

The answer to most of these questions are not available because of total neglect in this area of research. Even documentation of claims has not received sufficient attention except for stray attempts by Waheed and Siddiqui (1961); Nadkarni (1976); Chopra et al (1982); Murthy (1983); Dash (1986) etc. Some standardization work on Bhasmas and Kushtas has, however, been done under the auspices of the Central Council for Research in Ayurveda and Siddha and Central Council for Research in Unani medicine (Anonymous 1991). The ICMR and CCRUM have recently initiated some projects on gold, silver, arsenic and lead preparations used in Ayurveda and Unani-Tibb at the Department of Medical Elementology and Toxicology, Faculty of Science, Jamia Hamdard, New Delhi. This study is part of one such project sponsored by the ICMR.

2. Ayurvedic Gold Preparations

Swarna Bhasma (SB)

2.1 Methods of Preparation

Ancient Ayurvedic text describe several methods of preparation of Swarna Bhasma. These processes involve intimate mixing of gold leaves with mercury, sulphur, citric acid and the mixture is subjected to heat repeatedly using cow dung cakes as fuel. Calcination with herbal juices is usually done in earthen crucible sealed with clay soaked cotton bandages. Repeated ashing and trituration is believed to detoxify the metal. Some of the adjuncts used in these procedures includes;
- Oil
- Whey
- Cow’s urine
- Kanji
- Extract of *Dolichous uniflorous* (KULATHA KALAI)
- Citrus limon

Chopra and co-workers (1982) summarized some of the Ayurvedic methods as follows:

a) In reducing gold on part of the purified metal and two parts of mercury are rubbed with an acid and made into a ball. Powdered sulphur equal in weight of the ball is taken, half of the sulphur is placed in an earthen plate, the ball is placed over it and it is covered with the remaining half of the sulphur. The plate with its contents is covered with another earthen plate. A piece of rag is then smeared with clay and it is wrapped round the plate and dried in the sun. It is then placed on 30 pieces of dry cow dung cakes and roasted. The process is repeated 14 times when the gold converted into the bhasma form and is ready for use.

b) Gold is reduced to a fine powder by rubbing with mercury and exposing it to heat in a covered crucible with the addition of sulphur. Two parts of mercury and one part of purified gold leaf are rubbed together into a mass with lemon juice and three parts of sulphur. The crucible is then covered and exposed to heat. The process of mixing gold with mercury and exposing the mixture so formed to heat is repeated 14 times when the gold completely loses its apparent metallic characters. Some are of opinion that gold should be rubbed with mercury when roasted for the first and subsequent roasting should be done with sulphur alone.
c) Another process of preparing reduced gold is that gold is melted and its own weight ash of mercury is thrown into the molten metal. When cooled the mass is powdered and rubbed with lemon juice and cinbar and again roasted in a covered crucible. The process is repeated several times.

2.2 Physical Characteristics

_Swarna Bhasma_ is grey or brown coloured amorphous powder with a metallic taste. It is insoluble in water and contains gold in the form of an unidentified complex.

2.3 Medicinal Properties and Therapeutic Uses

It is attributed with tonic, rejuvenating and detoxicant properties and is claimed to be of value in infectious and neuropsychiatric ailments, general and sexual ability. The indications include anaemia, dyspepsia, epilepsy, neurasthenia, loss of memory and concentration, hysteria, schizophrenia, bronchitis, asthma, tuberculosis, cancer, impotency, looseness of bowels, epigastic pain, leucoderma and for the improvement of complexion (Chopra et al 1982; Dash 1986).

2.4 Chemical Composition

Chopra and coworkers (1982) analysed a sample of _Swarna Bhasma_ obtained from M/s Kalpataru Ayurvedic Works, Calcutta. Their findings are given in Table 2.2:
Table 2.2: Chemical composition of *Swarna Bhasma*

<table>
<thead>
<tr>
<th>Chemical Ingredients</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold, metallic</td>
<td>96.760</td>
</tr>
<tr>
<td>Silica (SiO₂)</td>
<td>1.140</td>
</tr>
<tr>
<td>Iron (Fe₂O₃)</td>
<td>0.140</td>
</tr>
<tr>
<td>Lime (CaO)</td>
<td>0.546</td>
</tr>
<tr>
<td>Copper</td>
<td>Traces</td>
</tr>
<tr>
<td>Magnesia</td>
<td>Traces</td>
</tr>
<tr>
<td>Phosphates (P₂O₅)</td>
<td>0.781</td>
</tr>
<tr>
<td>Potash</td>
<td>0.161</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.078</td>
</tr>
<tr>
<td>Sulphates</td>
<td>0.150</td>
</tr>
<tr>
<td>Moisture</td>
<td>0.244</td>
</tr>
<tr>
<td>Total</td>
<td>100.000</td>
</tr>
</tbody>
</table>

2.5 *Doses*

10-25 mg, twice daily. This may be administered with butter, cream, milk or ghee. In different disease conditions, it is used along with different vehicles.

2.6 *Adverse Effects of Impure Gold*

Gold, if used without proper purification may be harmful. It is stated to reduce the strength and intellect of individual (Dash 1986).
3. Unani Gold Preparations

*Kushta Tila Kalan* (KTK)

3.1 Method of Preparation

This gold preparation is used in the Mohammedan medicine. The method involves thorough trituration with mercury and sulphur and repeated calcination in sealed earthen containers. This mass is subjected to ashing using cow's dung cakes as fuel and then pulverized with juices of *Aloe vera* (GHEEKANWAR) and distillate of roses.

3.2 Physical Characteristics

KTK is greyish amorphous powder with a metallic taste. It is insoluble in water.

3.3 Medicinal Properties and Uses

The colloidal form of gold is claimed to be therapeutically useful in cases of alcoholism, neurasthenia, epilepsy. It is also attributed with nervine tonic, hepatic anti-infective, detoxicant and powerful sexual stimulant and to act beneficially in impotency. It has also been used in excessive nocturnal emission in those who masturbate. The other indications include antidote to poisons particularly those of bacterial origin, chronic fevers, gastrointestinal disturbances etc. (Chopra et al 1982).

3.4 Chemical Composition

Chopra and coworkers (1982) examined and analysed the specimen from well known Hakim of Delhi and quantitative composition is shown in Table 2.3:
Table 2.3: Chemical Composition of *Kushta Tila Kalan*

<table>
<thead>
<tr>
<th>Chemical Ingredients</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold, metallic</td>
<td>86.14</td>
</tr>
<tr>
<td>Other inorganic constituents</td>
<td>13.86</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
</tbody>
</table>

3.5 *Dose*

15-30 mg (Anonymous 1991)