CHAPTER 7

DISCUSSION
The perusal of the result revealed encouraging analgesic, anti-cataleptic, nootropic, antidepressant, anxiolytic, anti-aggressive, growth stimulating, glycogen sparing, adaptogenic and immunomodulatory effects of gold preparations studied.

1. Analgesic Actions

The test drugs (SB and KTK, 12.5 - 50 mg/ kg po, - 60 min) exhibited moderate to marked analgesic effects against varied stimuli (chemical, electrical, thermal and mechanical) in mice and rats. AN (2.5, 5mg/kg po) showed similar effects in all parameters except in tail clip test. The effects were found to be dose-dependent (except for AN) and compared well with the standard drugs (morphine and acetyl salicylic acid). The effects of morphine and the test drugs SB and KTK were partly blocked by pre-treatment with naloxone indicating involvement of opioidergic receptors in their mechanism of analgesic action. Unlike SB and KTK, AN showed no naloxone antagonism at the doses used. Combined treatment with morphine and the test drugs elicited some additive effects without evidence for a synergistic action. Gold is known to possess anti-inflammatory and anti-arthritis properties (Leibfarth and Persellin 1981). While there is no evidence to attribute the analgesic effects of these preparations exclusively to gold, it is interesting to note that the activity approximately paralleled their gold content (AN 29%, SB and KTK: approx. 47%). This aspect warrants further investigations keeping in view possible addition of unknown components from herbal juices during the calcination process. Pain is an important feature of inflammation. Our findings revealed good analgesic activity in the gold preparations as an independent entity rather than as a component of its reported anti-inflammatory action. Such activity is being reported for the first time in gold. The test drugs, therefore, may be classed as
opioidergic analgesics. Their potentiating effects on barbiturate-induced narcosis and naloxone antagonism supports this contention.

The elevated concentrations of several trace elements such as copper, molybdenum, manganese, tin, barium and cesium, reported in sera of the patients with rheumatoid arthritis, show a decline following gold therapy. It has been postulated that gold compounds might displace certain trace metals in combination with tissue or plasma proteins. The alterations could not be correlated to the clinical response and might well be secondary to decrease in inflammation (Insel 1996). No reports are available for such changes in trace element concentrations in pain per se or following analgesic therapy. Involvement of such mechanisms in the analgesic action of the test drugs is, therefore, not known. It is concluded that Ayurvedic and Unani gold preparations, under study, posses moderate analgesic properties elicited through opioidergic mechanism.

2. Anticataleptic Activity

All of the gold preparations revealed marked anti-cataleptic actions. The effects, which were seen within 40 minutes and lasted for more than 140 minutes, compared well with those produced by L-dopa. This is quite interesting in view of therapeutic relevance of such effects in Parkinson’s disease. Gold compounds are attributed with various medicinal properties and utility in neuropsychiatric disorders in Ayurveda and Unani-Tibb (Said 1969; Chopra et al 1982; Kabeeruddin and Wahid 1992). The corresponding terms for specific neuropsychiatric diseases are not available in Indian systems of medicine. Various terms such as Parkinson’s disease, schizophrenia, epilepsy, mania etc. are generally clubbed together and described as nervine weakness and lunacy and the
intended drugs as nervine tonics. DA depletion is known to cause catalepsy and Parkinson’s syndrome (Verma and Kulkarni 1992). The test drugs caused elevation of DA concentration in various areas of the brain. This indicates involvement of dopaminergic system in mediation of such effects.

3. Nootropic Effects

The gold preparations studied revealed favorable actions on cognitive functions both in the active and passive models of learning. Participation of multiple neurotransmitter and receptor mechanisms is well known in learning and memory (Kulkarni and George 1999). Enhancement of cholinergic neurotransmission is reported to facilitate learning and memory (Bartus et al 1982; Biegon et al 1986; Sudha et al 1995). The cholinergic concept is one of the principal theory in dementia associated with most of neurodegenerative disorders (Fibiger 1991). The learning of passive avoidance task (inhibitory) is processed by the cholinergic synapse (Sala et al 1991). The agents caused marked reduction in the activity of acetylcholinesterase (AChE) in frontal cortex and hippocampus region. AChE is a well known marker enzyme for cholinergic function. Decreased AChE activity, caused by treatment with gold preparations, possibly lead to increased ACh levels in brain thus producing improvement in cognitive functions. Beside ACh, other neurotransmitters eg. DA, 5-HT and NE are capable of modulating long-term potentiation (LTP) in the dentate gyrus (Bliss et al 1983). Opioid antagonists naloxone and nootropics, piracetam and aniracetam have also been shown to reverse the effect of scopolamine (Rush 1986; Vogelsang and Piercy 1986). It is interesting to note that test drugs are opioidergic analgesics with nootropic effects. Noradrenergic pathways are
associated with alertness and attention (Role and Kelly 1991). The test drugs elicited increase in NE levels on various regions of brain. Increased turnover of DA, observed in the study, could also play a role in nootropic effects shown by the test drugs. It is difficult to pinpoint precise mechanisms responsible for nootropic actions.

4. Antidepressant and Anxiolytic Actions

The gold preparations decreased immobility time in the forced swimming test and reversed the increase in shock-induced escape failures in the learned helplessness test indicating antidepressant activity. The most consistent finding concerning the neurotransmitter system in this context is diminished concentration of 5-HT or its metabolite 5-hydroxyindole acetic acid in depressed and suicide-prone patients (Mann et al 1989).

The test drugs elicited anxiolytic action as evidenced by increased punished drinking episodes, more number of entries and time spent in open arm and reduction in behavioural deficit. Van Praag (1998) presented the concept of serotonin-related anxiety and/or aggression-driven, stressor-precipitated depression. Anxiety is a core constituent of stress syndrome thus the serotonergic disturbance induced heightened sensitivity to stressful events i.e. the latter will induce stress phenomenon including anxiety and anger readily than normal. Increase in serotonin levels, following treatment with gold preparations, points to the probable mechanism of antidepressant and anxiolytic actions. Facilitation of central dopaminergic action may also contribute to such effects. The relation between stress and brain catecholamine levels is controversial. Restraint stress has been reported to enhance brain NE and 5-HT concentrations (Bhattacharya and Sur
1999; Derclanko and Long 1998). Paradoxically a new anxiolytic drug Ipsapirone is reported to stimulate catecholamine turnover in various regions of rat brain (Golimbiowska 1990). Whether increased catecholamine levels “observed by us” is relevant in this context needs to be looked into it.

5. Anti-aggressive Effects
Calcined gold preparations (KTK and SB) exhibited serenic (anti-aggressive) activity in isolation, electroshock and apomorphine-induced aggressive behaviour in rats and mice. Such activity was not observed in AN in any of the models at the doses used. It is predicted that serenics (like anxiolytics) act via normalisation of serotonergic circuits (Van Prag 1998). Matto and co-workers (1998) opined that blockade of 5-HT reuptake has no major influence on apomorphine induced aggressive behaviour, but the 5-HT1A receptors subtype may be involved in the mediation of aggressive behaviour in this paradigm.

6. Conflicting Conjoint Psychopharmacological Action
It will be seen that some of the psychopharmacological actions, observed with gold preparations (which are not purified compounds) in the present study, appear to be conflicting e.g. analgesic effects were found to be mediated through opioidergic mechanism but this does not conform to the observed anti-cataleptic action which represents inhibition of this system. Besides while the anti-cataleptic and the antidepressant activities suggest facilitation of central dopaminergic system, the observed anti-anxiety effect can not be rationalised on this premise. In addition the central
opioidergic system is well known to inhibit central dopaminergic activities and opioidergic receptors are known to exist on dopaminergic neurons. Such contradictions may be explained in the light of following facts:

(a) Morphine/pethidine are used as pre-anaesthetic agents to alleviate anxiety/apprehension associated with surgery (Hardman et al 1996).

(b) Opioidergic analgesics are known to produce euphoria, tranquility and alterations in mood. The mechanisms for these effects are not entirely clear. Microinjections of µ opioids into the ventral tegmentum activates dopaminergic neurons that projects into the nucleus accumbens. This pathway is postulated to be a critical element in opioid-induced euphoria (Hardman et al 1996).

(c) Buspirone (an anxiolytic agent) displays properties of both DA agonist and antagonist. Contradictory reports of its effects on DA synthesis (enhancement and reductions) are available. It has minimal activity on postsynaptic dopaminergic receptors (Jann 1988).

(d) The involvement of opioidergic system in the antinociceptive mechanism of antidepressant compounds has recently been reported (Gray et al 1998). These findings are consistent with the view that antidepressants may induce endogenous opioid peptide release. The authors demonstrated shift of dose response relationship to the right for antidepressant agents (dothiepin, amitriptyline, paroxetine etc.) following pre-treatment with opioid antagonist (naloxone and naltrindole). This implicates delta opioid receptor and endogenous opioid peptides in antidepressant-induced antinociception (Gray et al 1998). Various clinical and experimental reports also indicate that tricyclic antidepressant drugs are useful in the treatment of acute and chronic pain conditions via the
participation of endogenous opioid system and partly by further activating noradrenergic and serotonergic pathway (Valverde et al 1994).

(e) Animal models of depression have certain limitations as they are based on ability to support animal behaviour in stressful situation that ordinarily lead to decreased responsiveness (learned helplessness). Blockade of DA in these models is associated with their stimulant rather than antidepressant activity (Hardman et al 1996).

7. Glycogen Sparing and Anti-fatigue Action

Fatigue is closely linked to depletion of glycogen stores, both in skeletal muscle and liver tissue. This in turn affects the ability of work/performance. An animal, which can conserve body carbohydrate stores better, should be able to exercise longer at a given work and intensity than the animals that must rely upon muscle glycogen for contractile energy (Avakian et al 1979). In the present study, the two hour swimming exercise bout proved to be of sufficient intensity to deplete muscle and liver glycogen content. Treatment with gold preparations (KTK and SB) for 10 days reversed this and restored the liver and muscle glycogen content to near normal levels indicating glycogen sparing effects. Similar results have been reported with reputed adaptogen and rejuvenating plant drug *Panax ginseng* (Kumar et al 1996; Mitra et al 1996). KTK and SB treatment also showed restorative action on adrenal cholesterol content in exercised rats whereas AN failed to elicit such effects. Adrenal ascorbic levels were not influenced in exercised rats by any of the test drugs. These findings and increased activity counts point to anti-fatigue property in KTK and SB (but not in AN).
8. Growth and Body Weight

While there is general apprehension about the ill effects of heavy metals, it is interesting to note that incorporation of the test drugs in the diet of growing rat pups (0.01% w/w for 4 weeks) revealed significantly higher growth rate in treated animals with better efficacy in calcined preparations (KTK and SB) vs AN.

9. Antioxidant Effects

Incomplete reduction of molecular oxygen is known to generate free radicals, cause oxidative stress and enhance lipid peroxidation (Halliwell and Gutteridge 1984). These effects have been implicated in a wide range of human ailments including cancer, cardiovascular diseases, arthritis, diabetes, neurodegenerative disorders, aging etc. (Schaur et al 1991). The test drugs revealed antilipidperoxidative and antioxidant effects through alterations in antioxidant enzymes: Catalase, GSH, GST, GPD, GR. Such effects have been reported in AN and gold sodium thiomalate which inhibited production of reactive oxygen species and OH generation (Miyachi et al 1987). No reports are available on this aspect for KTK and SB.

10. Adaptogenic Effects

Calcined gold preparations (KTK and SB) almost normalized milk-induced leucocytosis in rats indicating a favorable effect on non-specific resistance of the body. The adaptogenic action, though of lesser magnitude, was observed at 25 mg/kg vs Panax ginseng which was used 350 mg/kg. The adaptogenic affects of this plant are well documented (Avakian et al 1979). AN revealed no adaptogenic actions.
11. Immunomodulatory Effects

Heavy metals can elicit both the immunosuppressive e.g. cobalt, cadmium, lead, nickel etc. (Koller and Roan 1977; Bozelka and Burkholder 1979; Karkvliet et al 1980; Lawrence 1981) and immunostimulant effects e.g. zinc (Singh et al 1992). Varied effects have been observed for different gold preparations (Davis and Johnston 1986; Hashimoto et al 1992). These reports indicate that the response induced by gold largely depends on the form in which it is presented. It is interesting to note that while AN elicited immunosuppressive effects, traditional gold preparations (KTK and SB) showed immunostimulant actions on various parameters of immune functions; the optimal dose for such effects was found to be 25 mg/kg. Higher doses of both the drugs elicited an immunosuppressive action. Histological examination of various vital tissues (kidney, liver, spleen, thymus) revealed no apparent toxicity of KTK or SB; moderate toxic manifestations were, however noted in some tissues at higher doses. Results of cellularity of lymphoid organs also showed a similar pattern. Specific immune functions (cellular and humoral immunity) as well as non-specific immune functions were modulated by KTK and SB. When non-specific immunity was measured in tumour challenge study, both the drugs showed inhibitory response. Gold preparations used in modern medicine e.g., auranofin, gold sodium thiomalate, gold sodium thioglucose and disodium aurothiomalate are known to have inhibitory action on B-cell, T-cell and macrophages functions in vitro and in vivo (Hassan et al 1986; Hashimotto et al 1992). Such effects, however, render them very useful in the therapy of autoimmune diseases especially, rheumatoid arthritis (RA). The activation of T-lymphocytes in particular is implicated in RA. The therapeutic agents, which modulate the production of the lymphocytes or their
response to interleukin-2 (IL-2), may alter chronic rheumatoid synovitis (Wolf and Hall 1988).

The cytotoxic effects of heavy metals have been attributed mainly to their immunosuppressive action. In the present study KTK and SB, exhibited stimulatory effects on cellular and humoral immune functions with no apparent cytotoxicity on different lymphoid cells up to an optimum dose of 25 mg/kg. Toxic effects, observed at higher doses, may be due to the chemostatic effect of heavy metal. Preparation of these drugs involves specialized techniques of calcination and trituration both in Ayurveda and Unani systems. Herbal juices eg. Aloe vera and Rosa damascena are also added during the ashing process (Bajaj and Vohora 1998). It is, thus, a mixture of varied herbomineral components with gold in the highest concentration. Aloe vera ingredients have been reported to possess immunomodulatory effects (Zhang and Tizard 1996).

Stimulatory effects were also observed on macrophage functions; AN revealed a suppressive effect. This is not surprising because suppressive effects of AN on immunocompetent cells are well documented (Hassan et al 1986; Wolf and Hall 1988; Hashimoto et al 1992; Sfikakis et al 1993). Auranofin is also known to inhibit phagocytosis in vitro (Davis and Johnston 1986). Bloom and coworkers (1988) reported that AN and gold sodium thiomalate did not exhibit any significant effect on immune function following a long-term regimen in dogs.

In immune system, cells and effector molecules work in a close co-ordination and macrophages constitute an important cell type involved in the initiation of many immune reactions (Mills et al 1976; Rosenstreich et al. 1976; Tagliabu et al 1979). Activation of such reactions may help the host to effectively neutralize the infection and tumour

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challenge. We demonstrated tumour growth inhibitory activity with KTK and SB in Ehlrich’s ascites tumour challenge test. Such observations may largely be due to stimulation of macrophage function by the test drugs as macrophages play an important role in tumour destruction (Alexander 1967; Evans 1973; Mannel et al 1980). Possibly the presentation of the metal to the cell in a fine emulsified form results in the stimulation of macrophages. While the precise changes responsible for transforming gold into nontoxic forms are not known, the present findings indicate that the immune response were modified in a positive manner by specialised Ayurvedic and Unani techniques used in preparation of SB and KTK.

12. Clinical Relevance
The question may be posed: Would expensive and potentially hazardous gold preparations be ever acceptable to clinicians who have a wide array of relatively safer and much cheaper anxiolytic, antidepressant and anti-cataleptic drugs at their disposal? Though animal models have their limitations in simulating aberrations of the human mind, these findings are very interesting and being reported for the first time in gold preparations, used both in traditional or modern systems of medicine. The effects compared well with standard drugs currently used for the purpose viz. diazepam, buspirone, imipramine and levodopa in various animal models. These drugs, despite being effective and fairly safe, are known to have some undesirable features e.g. sedative, amnesic, ataxic, tolerance and physical dependence-inducing actions of benzodiazepines (Hanlon 1996), pro-convulsant actions of buspirone (Vohora and Pillai 1998), anticholinergic effects and drug interactions (with antihypertensive and CNS depressants)
associated with imipramine, and postural hypotension and choreoathetosis with levodopa (Hardman et al. 1996). We do not claim superiority of the test drugs over conventionally used anxiolytic, antidepressant and antiparkinson drugs. It is, however, emphasized that search for newer psychotropic agents must continue from all sources including the Nature’s bounty: plants and mineral elements.

13. Safety Aspects

Are these agents safe? Gold therapy is known to elicit immunosuppressive effects, skin reactions, encephalitis, peripheral neuritis, nephrotoxicity, hepatotoxicity, cytotoxic effects, bone marrow depression and blood dyscrasia (Turner 1965; Leibfarth and Persellin 1981; Tozman and Gottlieb 1987; Felson et al. 1992; Tomioka and King 1997). In the present study most of the effects of KTK and SB were observed at 25 mg/kg, po (range 12.5 to 50 mg/kg, po). The MTD were found to be more than 2 g/kg, po (40-80 x ED), no weight loss or rota rod fall out was observed up to 1 g/kg, po (20-40 x ED). AN was used at 2.5 – 5 mg/kg. This drug revealed no mortality and untoward effects up to 100 mg/kg, po (20-40 x ED). Besides, no ill effects were observed on gross observation or in haematological parameters following single or multiple (10 days) doses. These observations suggest a wide range of safety for the test drugs. The preparations, under study, are not gold salts but calcined preparations of gold used in Ayurveda (SB) and Unani-Tibb (KTK) and involve incorporation of herbal juices (Aloe vera, Dolichos uniflorus, Rosa damascena), minerals (mercury, sulphur) and animal origin ingredients (Whey, cow’s urine) during the ashing process (Said 1969; Chopra et al. 1982; Kabeeruddin and Wahid 1992). They constitute unidentified complexes of the metal.
which may not have properties and biological effects akin to gold salts. There is some justifiable apprehension about the addition of mercury, most of the volatile metal is lost during the calcination process. The mercury content in the final products was found to be 0.10 - 0.15 ppm.

Long term studies were not done at this stage. Incorporation of gold preparations (0.01% w/w) into the diet of young rat pups for 4 weeks rather lead to higher growth rate in the treated animals vs the control group and AN-treated group. This is quite interesting and points to their tonic, rejuvenating properties as claimed in Indian systems of medicine. The use of these preparations in pregnancy, lactation (Anderson 1977) and in patients with concurrent renal and hepatic dysfunction, however, calls for caution and should be subjected to similar contraindications as advised for AN and other gold preparations used in modern medicine for the treatment of rheumatoid arthritis.

14. Conclusions

Heavy metals are known only for their negative aspects and toxic effects. This study presents the positive aspect for gold which has not been looked into with the sole exception of its therapeutic use in rheumatoid arthritis. Neurobehavioural, antioxidant and immunomodulatory effects shown by test drugs without discernible toxicity are quite encouraging. An overall improvement in physical (growth promotion, anti-fatigue) and mental (analgesic, nootropic, anti-cataleptic, anxiolytic and anti-depressant) performance and ability to cope up with stress, toxicity and infection (anti-oxidant, adaptogenic and immunomodulatory effects) is discernible. The preparations deserve more scientific attention with a dispassionate approach to explore their therapeutic potential. The
apprehension about the toxic and hazardous effects of gold preparations hindered progress in this area of research. The attitudes are fast changing towards a more balanced and dispassionate approach with the discovery of essential functions of arsenic and selenium (Friedon 1984), hitherto known only for their toxic effects.