Indian Journal of Pharmacology 2000; 32: 339-346 **RESEARCH PAPER**

ANTI-CATALEPTIC, ANTI-ANXIETY AND ANTI-DEPRESSANT ACTIVITY OF GOLD PREPARATIONS USED IN INDIAN SYSTEMS OF MEDICINE

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SUMMARY

Objectives: To study traditional gold preparations for anti-cataleptic, anti-anxiety and anti-depressant effects.

Methods: *Swarna Bhasma* used in Ayurveda, *Kushta Tila Kalan* used in Unani-Tibb and Auranofin used in modern medicine were subjected to videopath analyzer, vogel conflict/anxiometer, elevated plus maze, and social behavioural deficit tests for anxiolytic activity, behavioural despair and learned helplessness tests for antidepressant activity, haloperidol-induced catalepsy tests for neuroleptic activity, and maximum tolerated dose, gross behavioural observations and hematological parameters for safety evaluation in rats and mice.

Results: The test drugs caused significant increase in punished drinking episodes in anxiometer and open arm entries and time in elevated plus maze and decrease in behavioural deficit. A decrease in immobility time in forced swimming test, normalization of shock-induced escape failures in learned helplessness test, and reduction of haloperidol-induced catalepsy scores were also noted in treated animals. The maximum tolerated doses were found to be more than 80 times the effective doses and no weight loss or untoward effects were observed on gross behaviour and hematological parameters.

Conclusions: Traditional gold preparations used in Ayurveda and Unani-Tibb exhibited anxiolytic, antidepressant and anticataleptic actions with wide margin of safety.

KEY WORDS Gold Ayurveda Unani-tibb anxiety depression catalepsy

INTRODUCTION

There are three principal systems of medicine practiced in India: Ayurveda, Siddha and Unani-Tibb. These systems utilize drugs of natural origin constituting plants, animals and mineral preparations. While research on medicinal plants has received considerable attention, the mineral preparations have relatively been neglected. Studies on the role of elements in health and disease have now become of global importance with spurt of research activity in the last two decades[1-3]. The indigenous systems use mineral preparations mostly in calcined forms : *Bhasmas* in Ayurveda, *Kushtas* in Unani-Tibb and *Parpams* in Siddha. The usage includes even those elements which are otherwise considered toxic and not administered internally in modern medicine. One such metal is gold. At the present state of knowledge, it is classified as a non-essential accumulative trace element with no known biological function[1]. Two gold preparations Ayurvedic *Swarna Bhasma* and Unani *Kushta Tila Kalan* are claimed to possess general tonic, hepatotonic, nervine tonic, cardiostimulant, aphrodisiac, detoxicant, anti-infective and anti-aging properties[4-6]. Traditional calcination techniques are specialized processes wherein herbal juices are incorporated during preparation of ash. It is claimed that these processes purify (detoxify ?) the metal and make it therapeutically effective and safe [5,6]. These claims have not been validated. In modern medicine gold compounds (*e.g.* Gold disodium thiomalate and Auranofin) have been used in the treatment of rheumatoid arthritis for more than 60 years with well documented effects on immune function[7,8,9]. It is felt that this precious metal is capable of altering various biological functions. Investigation on gold, therefore,

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appears to be a fruitful area of research, more so for indigenous preparations which is a virgin field. In earlier communications we reported marked analgesic (elicited through opioidergic mechanisms) and immunostimulant-effects in these preparations with a wide margin of safety[10,12]. This communication embodies their anticataleptic, antianxiety and antidepressant properties.

MATERIALS AND METHODS

Swarna bhasma (SB): The drug was procured from M/s Ayurveda Ras Shala, Pune, India. It is prepared, following the method of Ayurvedic texts as described by Chopra *et al.*,[5] Analysis of SB at Sri Ram Institute of Industrial Research, Delhi revealed the presence of 46.9% of gold and 0.15 ppm mercury.

Kushta tila kalan (KTK): The drug was procured from M/s Hamdard (Wakf) Laboratories, Delhi , India. It is prepared by methods described in Unani texts[4,6]. Analysis of KTK at Sri Ram Institute of Industrial Research Delhi, revealed the presence of 47.3% of gold and 0.10 ppm mercury.

Auranofin (AN): The drug, procured from M/s Alidac Genetics and Pharmaceuticals, Ahmedabad (Goldar tablets), is known to contain 29% of gold[13].

Animals : Wistar strain albino rats (100-200 g) and Swiss strain albino mice (15-30 g) were used. The animals were kept in groups of 8-10 in polypropylene cages (23 x 38 x 23 cm) housed in air conditioned rooms (23-30 ºC) and maintained on a standard pellet diet (Amrut Laboratory , rat and mice feed. Navmaharashtra Chakan Oil Mills Ltd., Pune) and water *ad libitum*. The experiments were performed in a noise-free atmosphere at temperatures ranging from 29 ºC to 32 ºC. Approximately equal number of male and female animals were taken in each group.

Drugs

Haloperidol, buspirone and l-DOPA (Sigma laboratories, USA), amphetamine (John Baker, USA), diazepam (Calmpose, Ranbaxy, Gurgaon) and imipramine (Depsonil, S.G Pharmaceuticals, Vadodra).

Anxiolytic activity

Videopath analyzer : Activity of rats placed in a square chamber (50x50x35cm) was monitored using a videopath analyzer (Coulbourne Instruments, USA) with a computerised printing facility. Effects of diazepam, amphetamine and test drugs were studied on behavioral parameters *e.g.* ambulation, rearing, wall hugging and stereotypy during an observation period of 25 min (computerized programme of 5 sessions, each of 5 min duration with no inter-session interval).

Vogel conflict test : The test was carried out in water-deprived rats (48 h) using an anxiometer (Columbus, USA). Drinking episodes and number of shocks were recorded in vehicle, buspirone and test drugs-treated animals[14].

Elevated plus maze test: The apparatus similar to that described by Pellow *et al.,*[15] was used. It consist of a plus shaped maze elevated 45 cm above ground level. It has two open (10 x50 cm) and two closed (10 x 50 x 40 cm) arms. The test rat was placed in the central square area (10x10cm) of the plus maze and time spent in open arm during a 10 min observation period was noted. Data for vehicle, diazepam and test drug treated groups were compared.

Social behavioural deficit: The method described by Frances[16] was used. It involves observation of escape attempts by isolated (for 7-9 days) and grouped mice placed under an inverted beaker and tested together. After 30 sec acclimatization period, number of escape attempts for 2 min were counted. Social behavior was calculated using the following formula:

$$\text{Behavioural deficit} = \frac{Eg - Ei \times 100}{Eg}$$

Where
Eg = number of escape attempts by grouped animals. Ei = number of escape attempts by isolated animals.

Behavioural deficit (%) for vehicle, diazepam and test drugs-treated animals were compared.

Antidepressant activity

Behavioural despair: The method described by Porsolt and coworkers[17] was used. A rat was placed in a chamber (diameter: 45 cm, height: 20 cm) containing water up to a height of 15 cm at 25 ± 2 ºC. The animal can not touch the bottom of the beaker with its hind limbs or tail or climb over the edge of the beaker. Two swim sessions were conducted, an

Table 1. Anxiolytic effects of gold preparations using vogel's conflict test in rats.

Treatment [a]	Number	
	Licks	Shocks
Control, 1% gum acacia (10 ml)	59.85 ± 6.64	2.85 ± 0.34
Buspirone (5 mg)	121.71 ± 6.60***	6.00 ± 0.30***
KTK (25 mg)	92.85 ± 9.87**	4.57 ± 0.48**
SB (25 mg)	83.71 ± 11.63*	4.00 ± 0.53
AN (2.5 mg)	82.28 ± 11.64	4.00 ± 0.57

KTK: *Kushta Tila Kalan*; SB: *Swarna Bhasma*; AN: Auranofin

[a] Doses were given per kg, po x 10 days except in case of buspirone where a single dose was administered. Observations recorded 60 min after the last dose.

*p< 0.05,** p< 0.01,*** p < 0.001 when compared to control group, n= 7, values are mean ± SEM.

Table 2. Effect of gold preprations on social behaviour deficit.

Treatment [a]	Escape attempts		Behavioral deficit (%)
	Grouped mice	Isolated mice	
Vehicle, 1% gum acacia (10ml)	17.42 ± 0.75	9.28 ± 0.60	46.72
Diazepam (2.5mg)	4.57 ± 0.61*	4.28 ± 1.18	6.34*
KTK (25mg)	17.42 ± 1.54	15.71 ± 1.47	9.81*
SB (25mg)	16.71 ± 0.80	15.85 ± 1.51	5.14*
AN (2.5mg)	14.14 ± 1.28	13.14 ± 0.70	7.07*

KTK: *Kushta Tila Kalan*; SB: *Swarna Bhasma*; AN: Auranofin

*p < 0.001 when compared to control, n = 7

[a] Doses expressed per kg po for 10 days except diazepam which was given ip in a single dose. Latency (Diazepam) : - 30 min, other treatments : - 60 min

initial 15 min pretest, followed by a 5 min test 24 h later. Treatments were given after the pretest session. The period of immobility (passive floating without struggling and making only those movements which are necessary to keep its head above the surface of water) during the 5 min test period were noted for vehicle, imipramine and test drug treated animals.

Learned helplessness: The method of Bhattacharya *et al.,*[18] was modified as follows. Male rats subjected to foot shocks (60 scrambled shocks, 15 sec duration, 30V every min) and control rats (placed in an identical chamber without shock for 1 h) on day 1, were tested for avoidance trials in Techno jumping box 1h after the last dose of test drugs or vehicle on day 10. The apparatus consists of two chamber-comparatments divided by a transparent perspex hurdle. Wire grid floors of any of the chambers (area: 30 cm x 30 cm) can be charged by appropriate grill selector switch. The escape door during the learning sessions was kept closed and opened during the avoidance trials. Thirty trials were given using the following cycle: buzzer 3 sec, shock if necessary 3 sec, total time 6 sec. Observations were taken for crossing over to the shock-free chamber on warning buzzer alone (conditioned avoidance) and for escape failures. Values for vehicle, standard (imipramine 16 mg/kg, *i.p.* 3 doses, -2h, -5h and -1h, testing on day 3) and test drugs-treated groups were compared.

Neuroleptic activity

Catalepsy : Effects were studied on haloperidol induced catalepsy in rats using a 0-3 point scale as described by Dandiya and Banerjee[19].

Safety evaluation

LD_{50} was not determined. The test drugs were administered to groups of 10 mice at a dose up to 2 g/kg, *p.o.* for SB and KTK and 200 mg/kg, *p.o.* for AN. The animals were observed for gross behavioral effects, weight loss and mortality upto a period of 14 days. Further group of 7 albino rats were given vehicle, test drugs for 10 days and hematological parameters (Hb, RBC, WBC, ESR, PCV) were recorded 1 h after the last dose on day 10.

Statistical analysis: All data were expressed as mean ± SEM. ANOVA followed by Dunnet's t test was applied for analysis of results.

RESULTS

Anxiolytic activity

Videopath analyzer : The test drugs (25 mg/kg, *p.o.*) and AN (2.5 mg/kg, *p.o.*) for 10 days revealed no appreciable effects on locomotion, rest, distance travelled, rearing, wall hugging, corner position and stereo events.

Table 3. Effect of gold preparations on immobility using behavioral despair test.

Treatment [a]	Immobility time for 5min (Mean ± SEM)
Control, 1% gum acacia, (10ml)	242.00 ± 4.07
Imipramine (16 mg)	134.25 ± 10.40*
KTK (25 mg)	151.42 ± 2.23*
SB (25 mg)	162.85 ± 4.86*
AN (2.5 mg)	171.42 ± 5.20*

KTK: *Kushta Tila Kalan*; SB : *Swarna Bhasma*; AN: Auranofin
* p< 0.001: Comparison vs control, n = 7
[a] Doses were given per kg po X 10 days except in case of Imipramine where three doses were administered ip at -24h, -5h and -1h.

Table 4. Effect of gold preprations on learned helplessness using rats.

Treatment *	Avoidance response (A)	Escape failure (N)
Vehicle control, 1% gum acacia (10ml) without shock	16.16 ± 0.65	4.66 ± 0.66
Vehicle control, 1% gum acacia (10ml) with shock	14.83 ± 0.74	9.66 ± 0.24 [a]
Imipramine (16mg)	21.80 ± 0.62 [b]	4.50 ± 0.75 [b]
KTK (25mg)	19.14 ± 0.71 [b]	4.37 ± 0.41 [b]
SB (25mg)	18.50 ± 0.46 [b]	4.00 ± 0.26 [b]
AN (2.5mg)	16.25 ± 0.54	6.50 ± 0.32

KTK: Kushta Tila Kalan; SB: Swarna Bhasma; AN: Auranofin
[a] p< 0.001: Comparison vs vehicle control without shock and
[b] p< 0.001: Comparison vs vehicle control with shock, n= 6-8
* Doses were given per kg po X 10 days except in case of Imipramine where three doses were administered ip at -24h, -5h and -1h.

Vogel conflict test : Buspirone (5 mg/kg, *p.o.*, single dose, -60 min), KTK and SB (25 mg/kg, *p.o.*, x 10 d, - 60 min) caused a significant increase in punished drinking; the number of licks and number of shocks were increased except for SB in which the observed increase was statistically significant only for number of licks. The elevated values for AN (2.5 mg/kg, *p.o.*, x 10 d, -60 min) also did not differ significantly from vehicle-treated animals (Table 1).

Elevated plus maze test : The results are shown in Figure 1. The test drugs and AN treated animals showed more number of entries and time spent in open arm when compared to vehicle treated rats. The effects were significant (p<0.001) and compared well with diazepam (2.5 mg/kg, *i.p.*, -30min). The combined treatment with diazepam and gold preparations showed more effect when compared to that elicited by either treatment alone. No synergism was observed.

Social behavioural deficit : Both the test drugs and AN caused significant (p<0.001) reduction in behavioural deficit at the doses used. The effects compared well with those elicited by diazepam (Table 2).

Antidepressant activity

Behavioural despair: All the drug treatment significantly (p<0.001) decreased the immobility time of rats subjected to forced swimming. The effects compared well with imipramine at the doses used (Table 3).

Learned helplessness: A significant increase in escape failure was observed in control animals subjected to shock when compared to those without shock (Table 4). Both the standard drug (imipramine) and gold preparations (SB, KTK and AN) revealed a reversal of such effect bringing the escape failure values to the level of untreated animals without shock. The effects were comparable to imipramine. The test drugs (SB and KTK) and imipramine also caused significant (p<0.001) increase in avoidance response. While such increase was also noted in AN treated animals, the difference was not found to be statistically significant (Table 4).

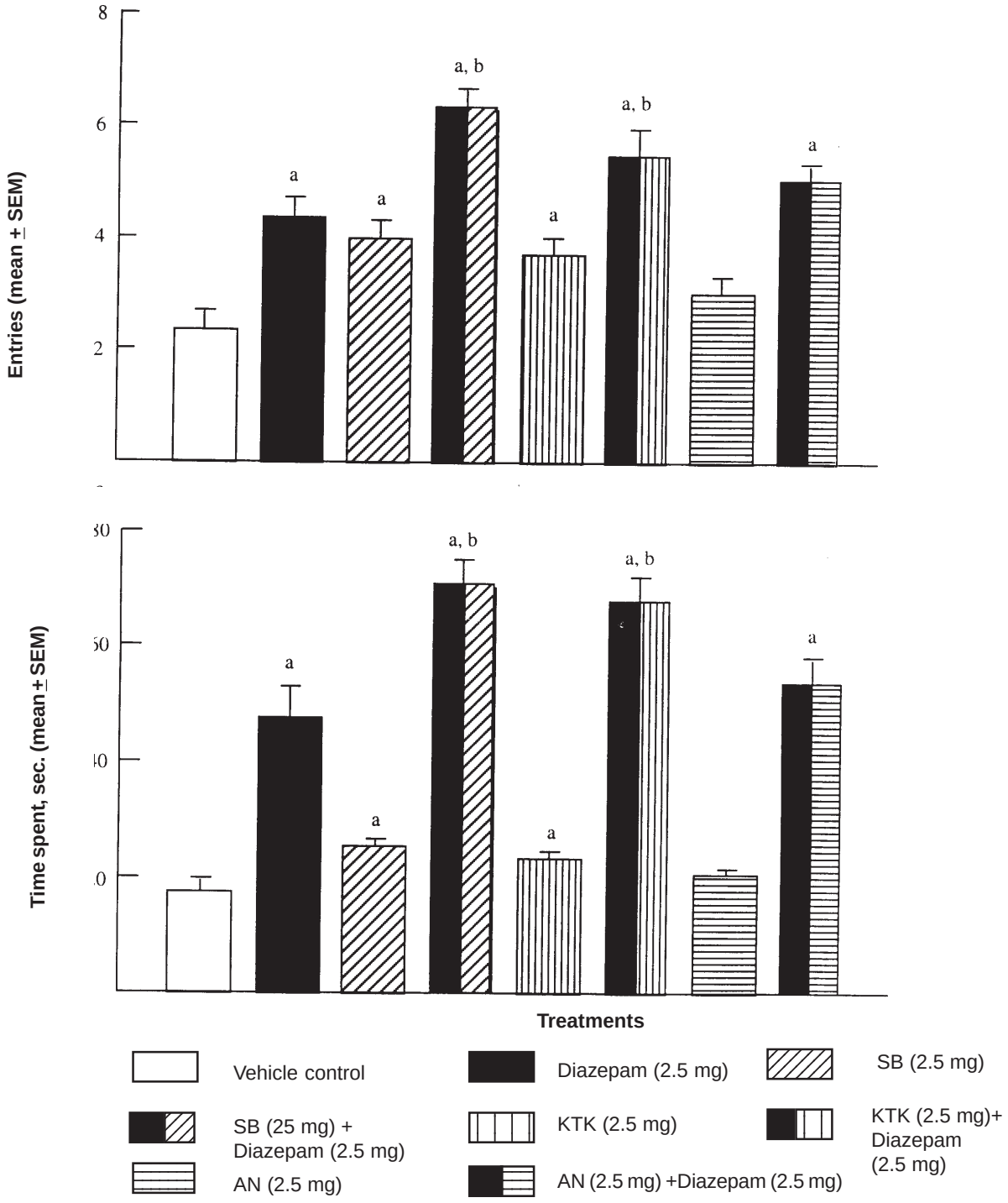
Anti-cataleptic activity

All of the gold preparations under study revealed marked anti-cataleptic actions ; the haloperidol- induced catalepsy scores were significantly lower ((p<0.001) in treated animals when compared to rats injected with haloperidol alone. The effects, which were seen within 40 minutes and lasted for more than 140 minutes, compared well with those produced by L-DOPA (150mg/kg, *i.p.*, -30min) (Figure 2).

Safety evaluation

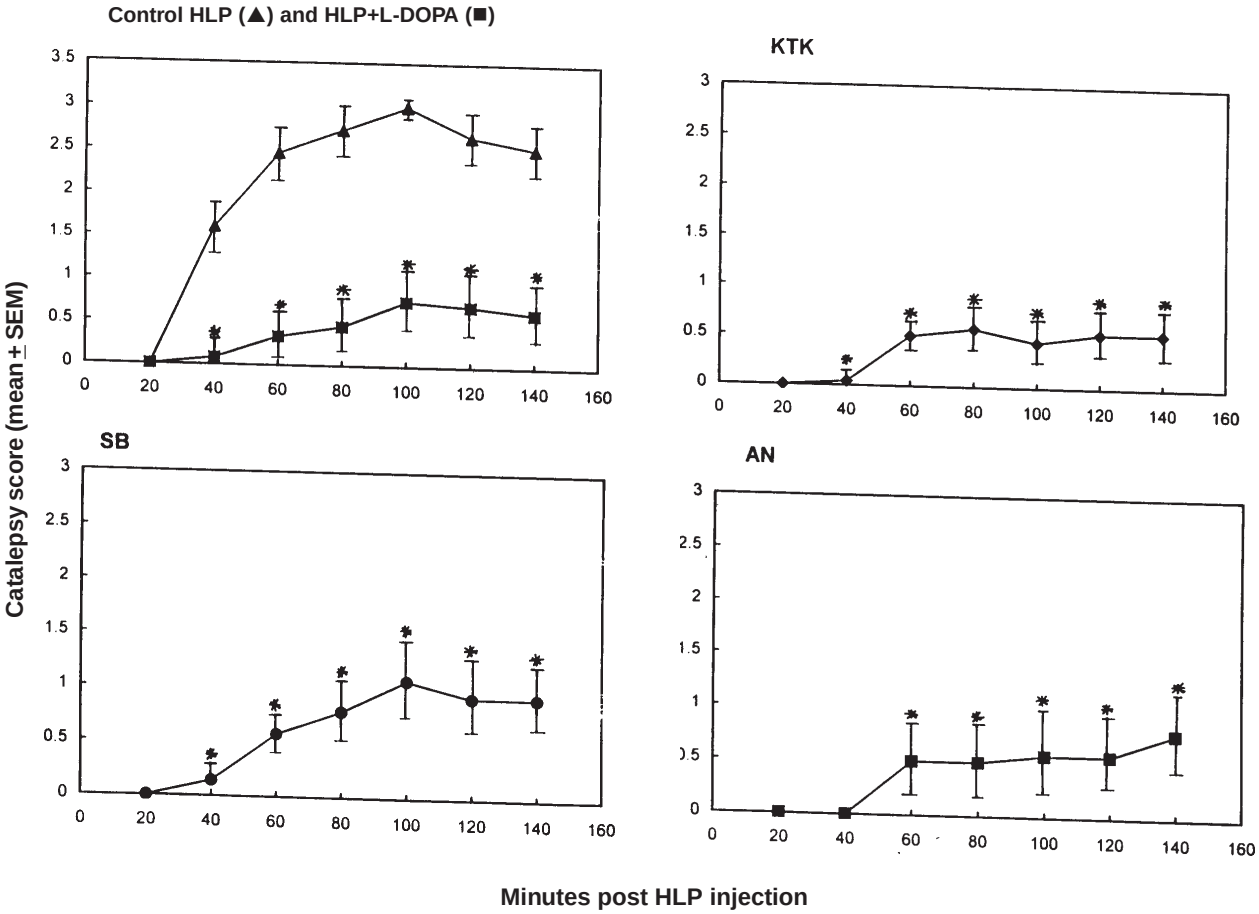
The MTD were found to be >2 g/kg, *p.o.* for SB and KTK and >200 mg/kg *p.o.* for AN. None of the test drugs treated animals exhibited mortality or weight

Figure 1. Anxiolytic effects of gold preparations using elevated plus maze test



a: Compared vs control, b: Comparisons vs Diazepam, p <0.001, n=7
Doses expressed per kg *p.o.* for 10 days except diazepam which was given *i.p.* in a single dose. Latency (diazepam) -30 min, other treatments-60 min. Observation time: 10 min

Figure 2. Effect of gold preparations on haloperidol induced catalepsy in rats.



* p <0.001; comparison vs control; n=6
HLP: 0.025 mg, L-DOPA: 150 mg, KTK:25 mg, SB:25 mg, AN:2.5 mg. All treatment were given per kg, *p.o.* for 60 min except HLP which was given *i.p.,* *p <0.001 when compared to control; n=6.

loss up to MTD's when observed for 14 days following single administration. No untoward effects were discernible when gold preparations were incorporated in diet at a concentration of 0.01% for 1 month. No hematological abnormalities were seen following 10 day treatment with the test drugs.

DISCUSSION

Anxiety, depression and mental health problems in general and senile neurological disorders in particular, are widely prevalent in modern fast-paced life with a multitude of stressful conditions. While gold is used only for the treatment of rheumatoid arthritis in modern medicine[7-9] it is attributed with varied medicinal properties in Ayurveda and Unani-Tibb including nervine tonic effects and utility in neuropsychiatric[4-6]. The corresponding terms for specific neuropsychiatric diseases (Parkinson's disease, schizophrenia, epilepsy, mania *etc*) are not available in Indian systems of medicine. These are generally clubbed together and described as nervine weakness, lunacy etc. These claims have not been subjected to scientific scrutiny.

The gold compounds under study SB, KTK and AN

exhibited anxiolytic, antidepressant and anti cataleptic actions with a wide margin of safety (MTD : > 80 times the test doses). 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[20], pro-convulsant actions of buspirone[21], anti-cholinergic effects and drug interactions (with antihypertensive and CNS depressants) associated with imipramine, and postural hypotension and choreoathetosis with levodopa[22]. 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.

Some of conjoint psychopharmacological actions observed with test drugs (which are not purified compounds) in the present study (anticataleptic, antidepressant and anxiolytic effects) and in our earlier report (analgesic actions through opioidergic mechanism)[10] appear to be conflicting but these should be viewed in the light of following facts: (a) morphine/ pethidine are used as pre-anaesthetic agents to alley anxiety/ apprehension associated with surgery[22]. (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[22]. (c) buspirone (an anxioselective 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[23]. (d) the involvement of opioidergic system in the antinocciceptive mechanism of antidepressant compounds has recently been reported[24]. These findings are consistent with the view that antidepressant may induce endogenous opioid peptide release. The authors demonstrated shift of dose response relationship to the right for antidepressant agents (dothiepin, amitriptyline, paroxetin *etc.*) following pre-treatment with opioid antagonist (naloxone and naltrindole). This implicates delta opioid receptor and endogenous opioid peptides in antidepressant-induced antinocciception[24]. 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[25], (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[22].

Are these agents safe? The adverse effects of gold salts particularly on prolonged use (nephrotoxic, bone marrow depression, cutaneous reactions and blood dyscriasis *etc.*) are well documented[9,26-28]. 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[4.5,6]. They constitute unidentified complexes of the metal which may not have properties and biological effects akin to gold salts. We reported immunostimulant, rather than immunosuppressant actions[28] and analgesic actions[12], in SB and KTK without descernible untoward effects at the doses used. The apprehension about the toxic and hazardous effects of the latter hindered progress in this area of research but the attitudes are fast changing towards a more balanced and dispassionate approach with the discovery of essential functions of arsenic and selenium[28] hitherto known only for their toxic effects.

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