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Effects of Some Indigenous Indian Medicinal Preparations on Cognitive Functions

Gyan Vikash Mishra* and S. B. Vohora#

*P G Deptt. of Zoology, Govt. College, Dungarpur (Rajasthan)

Deptt. of Medical Elementology and Toxicology
Hamdard University, New Delhi.

In Indian indigenous systems of medicines namely *Ayurveda* and *Unani-Tibb* some mineral preparations are used and called as *Bhasmas* and *Kushta* respectively. Preparations of these drugs involve calcination of certain metals and incorporation of some herbal juices into it through some exhaustive procedures. In the present study four such drugs have been evaluated for their toxic manifestations on cognitive functions. Two drugs are the calcined forms of mercury and two are of lead taken one each from Ayurvedic and Unani systems. Ayurvedic preparations were *Kajjali Bhasma* (Hg) and *Nag Bhasma* (Pb), and Unani drugs were *Kushta-Sangraf* (Hg) and *Kushta-Surb* (Pb).

For testing the cognitive activity two behavioural models of active and passive learning with retention were used in the study. To substantiate these findings neurochemical estimation of acetylcholinesterase and serotonin in discrete brain areas were also carried out. All the four drugs caused the impairment of cognitive functions along with corresponding effects on neurochemical parameters on high dose in ten days study. Result in this study calls for some extra precautions while prescribing such extensively used preparations.

Key Words: Kushta Sangraf (KS-I), Kushta Surb (KS-II), Kajjali Bhasma (KB), Nag Bhasma (NB), Learning Score, and Retention

Introduction:

Metals and minerals are being used in Indian indigenous systems of medicines (i.e Ayurveda, Unani-Tibb and Siddha) since over two hundred years. Metal products are meant both for external and internal uses and prescribed for treating obstinate and otherwise incurable conditions as well as in common ailments (Dash, 1986). Most of these preparations are basically 'HERBO-METALLIC' in nature. During the course of preparation metals are calcined and some herbal juices are incorporated. In Ayurvedic and Unani systems these preparations are called as *Bhasma* and Kushta, respectively.

* Corresponding author

These drugs are prepared from the metals like arsenic, mercury and lead, which are designated to be toxic on internal uptake by toxicologists and clinical experts. According to Ayurvedic literature metals like mercury and lead are subjected to physico-chemical processing called *Samskaras,* attributed to purification, detoxification and restoration of therapeutic properties (Dash, 1986). Unani philosophy states that in the course of preparation metal is **killed** and in its **body** the **soul** of herbal juices are incorporated (Bakht and Mahdihassan, 1984). However, such claims remain to be validated scientifically.

In this study four such drugs has been taken, two from Ayurvedic and two from Unani System. In each system one preparation was of mercury and another of lead. Host of literature is available to suggest the adverse neurological and psychiatric manifestations of these metals (Von Berg and Greenwood, 1991; Clarkson, 1998; Friberg and Nordberg, 1973; U.S.E.P.A., 1986; Skerfving, 1993). Impairment of cognitive function is also an implication prominently associated with these heavy metals (Laksmanna *et al*., 1993; Needleman, 1989). In the light of these informations, it becomes imperative to investigate the effects of these drugs on cognitive functions. These preparations are very oftenly used by **Vaids** and **Hakims** on general population, and the study employing modern scientific/ pharmacological tools for the toxic manifestations has not been conducted. Hence this study was undertaken.

Materials and Methods

Test Drugs - Unani drugs *Kushta Sangraf* (KS-I) the mercury preparation and *Kuhsta Surb* (KS-II) the lead preparation were procured from M/S Hamdard (Wakf) Laboratories, Delhi. Ayurvedic formulation of mercury *Kajjali Bhasma* (KB) manufactured by Zandu Pharmaceutical Works, Mumbai, and lead preparation *Nag Bhasma* (NB) of Shree Vaidyanath Ayurved Bhavan Ltd. Jhansi were purchased from market.

Animals - The investigations were carried out on adult animals [Wistar strain albino rats (100-200 g) and Swiss albino mice (30-50g)] of

either sex. The animals were kept in group of 8-12 housed in polypropylene cages kept in air-conditioned rooms (25-28°C) and maintained on standard pellet diet (Amrut Laboratory rat and mice feed, Navmaharashtra Chakan Oil Mills Ltd., Pune) and water *ad libitum.* Approximately equal number of male and female animals were taken in each group. The experiments were performed in a noise-free atmosphere at ambient temperature ranging between 24-32°C.

The test drugs (KS-I: 300 mg/kg, KS-II: 30 mg/kg, KB: 1000 mg/kg, NB: 1000 mg/kg, po) were administered in groups of 8-12 albino rats for 10 days. The control group received gum-acacia (1%: 10 ml/kg, po).

Behavioural testing :

1. *Active Avoidance Learning*

The apparatus described by Cook and Widely (1957) was used to study the efficacy or toxicity of the drug on acquisition of learning and its retention in albino rats. The scoring pattern described by Vohora *et al.* (1995) was followed. The animals were placed in the chamber for learning trials. A warning buzzer for 15 sec. was followed by shock (if needed). Climbing the pole was considered as a positive response. The maximum time allowed for each trial was 45 sec and thereafter the animal was removed from the chamber irrespective of the response. The responses were marked as follows: Avoidance response :

Climbing the pole without shock, Escape response: Climbing the pole after shock, and No response: No climbing upto 45 sec. A total number of 10 such trials were given. Three consecutive avoidance responses were considered as a criteria for acquisition of learning.

Twenty four hours later another trial was given to ascertain the retention of learning. Thus the observations included. (i) Acquisition (%), (ii) Mean learning score and (iii) Retention (%) in each group under study.

The scores 7 to 0 were recorded as follows:

Trials required	Score
2 - 4	7
3 - 5	6
4 - 6	5
5 - 7	4
6 - 8	3
7 - 9	2
8 - 10	1
> 10	0

2. *Passive Avoidance Learning*

The method used was similar to that described by Kulkarni and Verma (1992). Here we used the Continuous Avoidance Response Apparatus (Techno, Lukhnow, India) with an inverted petridish, which served as shock free zone (SFZ). The test mouse was placed on the grid floor and given electroshock (20 volts). The latency (time to reach the SFZ) was noted for each animal. Mouse exhibiting latency < 2 min were selected. After one hour the animals were tested again and number of the mistakes (climbing down in the shock zone), and time spent in SFZ (seconds) were observed for a period of 15 min.

Biochemical Estimations

Brain Sections - One hour after the last dose on day 10, the animals were sacrificed by cervical dislocation and the brains were quickly removed and placed in a brain block holder. Relevant brain regions *(i.e* hippocampus and frontal cortex) were dissected out following the method of Glowinski and Iversen (1966). It was ensured that while dissecting and weighing, tissues were kept on ice throughout.

Acetylcholinesterase (AChE) Activity- Analysis was done using the method of Ellman *et al.* (1961). The animals were sacrificed one hour after the last dose and samples from two rats were pooled and then processed following the method of Thakkar and Mallick (1993). Protein estimation was carried out by the method of Lowry *et al.* (1951).

5-Hydroxytryptamine (5-HT) Activity- Analysis was done using HPLC (High performance liquid Chromatography) with EC detector following the method of Saller and Salama (1984).

Results and Discussions:

Active avoidance learning score

High doses of all test drugs given for ten days, showed a significant lowering of learning score ($p<0.05$). In acquisition and retention also some significant lowering was discernible. (Table 1)

Table 1: Effect of Ayurvedic and Unani Mercury and Lead preparations on Active Avoidance Learning in Albino rats: Ten days treatment

Treatment	Dose (mg/kg, po)	Learning Score (Mean ± SEM)	Acquisition (%)	Retention (%)
Control 1% gum acacia	10 ml	5.39 ± 0.67	95	95
KS-I	300	2.80 ± 0.62*	60^{+}	60^{+}
KS-II	30	2.80 ± 0.52*	60^{+}	40^{+++}
KB	1000	3.28 ± 0.38*	80	50^{++}
NB	1000	2.82 ± 0.52*	70	30^{+++}

KS-I: *Kushta Sangraf*, KS-II: *Kushta Surb*, KB: *Kajjali Bhasma*, NB: *Nag Bhasma*
Comparison vs control group: * $p<0.05$ (t-test); + $p<0.05$, ++ $p<0.01$, +++ $p<0.001$ (x^2 - test), (n=10/20)
Chi-square value calculated on actual no. of animals.

Passive avoidance response

Treatment of the test drugs showed divergent effects: a significant increase in number of mistakes and time in shock zone by KB and NB and a non significant decrease in number of mistakes by KS-I and KS-II (Table 2)

Table 2: Effect of Ayurvedic and Unani preparations of Mercury and Lead on Passive Avoidance Learning in albino mice: Ten days treatment

Treatment	Dose (mg/kg, po)	Mistakes (no., Mean ± SEM)	Time in shock zone (sec., Mean ± SEM)
Control 1% gum acacia	10 ml	4.60 ± 0.45	28.98 ± 2.15
KS-I	300	3.80 ± 0.37	19.00 ± 2.80
KS-II	30	3.90 ± 0.19	33.40 ± 3.01
KB	1000	6.86 ± 0.52**	35.80 ± 1.91**
NB	1000	6.51 ± 0.31**	42.00 ± 3.21**

KS-I: *Kushta Sangraf,* KS-II: *Kushta Surb,* KB: *Kajjali Bhasma,* NB: *Nag Bhasma*
Comparison vs control group: ** $p<0.01$, (n=10)
Observation period - 15 min.

Biochemical Estimations

Acetylcholinesterase (AchE)- In 10 days treatment of all the test drug elicited significant reduction of AchE in frontal cortex and hippocampus of brain (Table 3).

5-Hydroxytryptamine(5-HT)- After the treatment of test drugs for ten days 5-HT levels in hippocampus was nearly same in KS-I treated group but showed elevation in groups treated with other three drugs (Table 4).

Table 3: Effect of Ayurvedic and Unani Mercury and Lead preparations on Acetylcholinesterase (AchE)activity in hippocampus and frontal cortex of rat brain: Ten days treatment

Treatment	Dose (mg/kg, po)	Acetylthiocholine hydrolyzed/mg Protein/min. (μM, Mean ± SEM)	
		Hippocampus	Frontal cortex
Control 1% gum acacia	10 ml	13.14 ± 1.25	9.48 ± 0.44
KS-I	300	8.80 ± 1.01*	7.44 ± 0.64*
KS-II	30	8.59 ± 1.20	6.97 ± 0.59**
KB	1000	8.90 ± 1.31	6.72 ± 0.86**
NB	1000	8.53 ± 1.16	7.06 ± 0.88*

KS-I: *Kushta Sangraf,* KS-II: *Kushta Surb,* KB: *Kajjali Bhasma,* NB: *Nag Bhasma*
Comparison vs control group: * $p<0.05$; ** $p<0.01$(t-test)

Table 4 : Effect of Ayurvedic and Unani Mercury and Lead preparations on 5-HT (5-hydroxytryptamine) activity in hippocampus of rat brain: Ten days treatment

Treatment	Dose (mg/kg, po)	5-HT in ng/gm of wet tissue in pooled samples (% increase ? / decrease ?)
		Hippocampus
Control 1% gum acacia	10 ml	546.87
KS-I	300	518.75 (5.14 ?)
KS-II	30	600.00 (9.71 ?)
KB	1000	714.06 (30.57 ?)
NB	1000	675.00 (23.42 ?)

KS-I: *Kushta Sangraf,* KS-II: *Kushta Surb,* KB: *Kajjali Bhasma,* NB: *Nag Bhasma*

Mercury has long been associated with the loss of memory and ability to concentrate (Friberg and Nordberg, 1973). Lead has also been linked to deficits in psychomotor intelligence, speech language processing, classroom performance and IQ of children (Needleman *et al.,* 1979; Bellinger *et al.,* 1986). Impairment of learning on exposure to lead during post-natal development of rats has also been reported (Kumar and Desiraju, 1992; Rice, 1992 and Altman *et al.* 1993). As pointed out earlier, reviews of Clarkson (1998) and Skerfving *et al.* (1998) also say a lot about the adverse effects of these two heavy metals on cognitive functions. No reports are however available about any of the behavioural effects of the indigenous preparations of the two metals tested here. But, these drugs are made up of the mercury and lead, and have shown to contain the inorganic forms of the two heavy metals as ingredients in respective formulations on Atomic absorption spectroscopy (Mishra, 2001). Hence, the metal content of these drugs seems to be causing this particular form of subtle toxicity, evidenced on both the active and passive models of learning and memory. Though, exceptions with KS-I and KS-II on passive model were encountered.

Cholinergic markers in areas of frontal cortex and hippocampus has been established to be associated with memory and cognitive functions (Flood *et al.* 1984; Gage and Bjorklund, 1986; Dekker *et al.,* 1991 and Bhattacharya *et al.,* 1995). According to Sudha and co-workers (1995) impaired learning and memory functions are associated with increased 5-HT levels and decreased AchE in hippocampus. Histochemical study by Biegon *et al.* (1986) also supports this assumption. Our results on biochemical parameters are also in agreement of two such possible mechanisms. AchE levels in hippocampus and frontal cortex has been shown to be down significantly in drug treated group of animals. Similarly elevated levels of 5-HT has been encountered in this brain area except for KS-I getting group. Enhanced synthesis of norepinephrine (NE) in *locus coeruleus* area has been linked to REM sleep deprivation (Siegal and Rogawski, 1988). Latter is believed to impair cognitive function (Crick and Mitchson, 1983; Christos, 1993). All these drugs cause the increased synthesis of NE in brainstem region (Mishra, 2001). This phenomenon may also be related to the said response. Learning impairment and other such effect is also said to be mediated by glutamate/ dopamine mesocorticolimbic system (Cory-Slechta, 1997), and significant alterations in DA level in

various brain areas following the treatment of these drugs has been reported by Mishra (2001). Hence to establish a possible mechanism for this effect of impairment of learning and memory by the test drugs is difficult. But, the most plausible reasoning comes from cholinergic alterations. Among the four drugs KS-I and KS-II did not produce the impairment of cognitive function on passive model. But, results on active model and AchE level in relevant brain areas does exhibit their potential for the said adverse response. These results certainly warrant a proper discretion in prescribing and medical surveillance while administering these drugs.

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