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Pharmacological Study

Evidence for safety of Ayurvedic herbal, herbo-metallic and *Bhasma* preparations on neurobehavioral activity and oxidative stress in rats

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Abstract

Heavy metals in Ayurvedic formulations have been used for centuries with claimed efficacy and safety. However, concerns are often raised about the toxicity due to heavy metals used in Ayurvedic formulations. The aim of present study is to explore the effect of Calcury tablet, Energic-31 capsule and *Basanta Kusumakara Rasa* (BKR) on neurobehavioral activity and oxidative stress in rats. Male wistar rats weighing 200-250 g were used and divided into normal control, positive control (mercury chloride, lead acetate, cadmium chloride, sodium arsenite, each 10 mg/kg, p.o for 28 days) and treated group (Calcury tablets at doses of 130, 650, 1300 mg/kg, Energic-31 capsule at doses of 150, 750, 1500 mg/kg and BKR at doses of 26, 130, 260 mg/kg, p.o. for 28 days). After performing the behavioural parameters on the 29th day, homogenate of rat's brain was used to determine malondialdehyde (MDA) and glutathione (GSH) levels and heavy metal level in brain. Results showed that there were no significant change in cognitive function, motor coordination, MDA and GSH levels as compared to normal control group at all doses of Calcury tablet, Energic-31 capsule and *Basant Kusumkar Rasa*. However, heavy metals level in rat's brain was higher as compared to normal control group at all doses of Calcury tablet, Energic-31 capsule and BKR. In conclusion, Calcury tablet, Energic-31 capsule and BKR in doses equivalent to the human dose does not have appreciable adverse effects on brain which demonstrates the non-toxic nature of metal based Ayurvedic formulations.

Key words: Ayurvedic formulations, neurobehavioral activity, oxidative stress, heavy metals

Introduction

In India, it has been estimated that about 14% sick persons utilizes Indian system of medicine. On the basis of preference, 18.7% population uses Ayurveda for normal ailments, 7.1% in case of sickness and 5% in case of serious ailments.[1] A report by the World Health Organization (WHO) indicates that many people in developing countries still rely on herbal medicine.[2] Majority of people believe that herbal medicines are safe and nontoxic, unlike modern chemotherapeutic agents. Individuals generally use herbal medicine for prolonged periods to achieve a desirable effect. On contrarily, it has been reported that herbal drugs used in the Indian subcontinent and China contain higher concentration of heavy metals than in other areas.[3-5] Another study showed that one of five Ayurvedic herbal medical products, produced in South Asia contains high levels of lead, mercury, and arsenic.[6,7] However, heavy metals are integral to some formulations and are been used for centuries.[8] Ayurvedic formulations are produced after different processes like detoxification, trituration and heating etc., of raw material. Therefore, elements present in finished products do not produce toxicity. Ayurvedic textbooks takes note of the toxicity of heavy metals and recommend special pharmacological process to detoxify them. Those metals which are obtained from ores may contain several impurities. These impurities are removed by *Shodhana* process. The *Shodhana* process removes unwanted part from the raw material and separate out impurities. In context of *Bhasma*, *Shodhana* means purifying and making the product suitable for the next step i.e. *Marana*. Ayurveda classifies *Shodhana* into (a) general process and (b) specific process. In general process for *Shodhana*, the sheets of metals are heated till red hot and are successively dipped into liquids like oil, buttermilk, cow's urine etc., and the procedure is repeated 7 times. In specific process

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for *Shodhana*, for some metals a specific process is described for *Shodhana* e.g. for purification of *Jasada*, the molten mass is poured in cow's milk for 21 times.[9] Ayurvedic text books emphasize the role of heavy metals in the proper function of the human body. In *Rasa Shastra*, the metals and the minerals are also termed as "*Dhatus*" and "*Updhatus*" because of their specific role in biological systems i.e. they can sustain body tissues by supplementing some of the essential elements to the tissues, whose deficiency causes many undesired problems or disease in the body. The available Ayurvedic literature emphasis the need of metals in maintaining the metabolic equilibrium of the human body. These metals are mercury, gold, silver, copper, iron, tin, lead, zinc etc., Any deficiency, excess or imbalance in the composition of these metals leads to certain metabolic and anabolic disorders. Equilibrium state of metals in the human body provides the basis for strong immunity.[10] Therefore, any imbalance in the composition of these metals could cause diseases and equilibrium of these metals is seen as a preconditioning for a normal immune defense and general health. Therefore, heavy metals from outside are deliberately added and processed with herbal plants to form herbo-metallic drugs.[11] Each time before burning, the metallic powders are processed with fresh herb juices to neutralize their toxicity. Some of the metals are burnt up to 100 times to make sure the heaviness or toxic effect of the metal is nullified. Once the "*Bhasma*" is ready it is tested for toxicity. One of the numerous tests the *Bhasma* has to pass through is called "*Varitar*" which means the *Bhasma*, once ready for internal use, floats on water indicating non-existence of heavy metal in it. The "*Bhasma*" are then transformed to compound formulas by mixing herbal powders. Special herbal juices are used for processing the compound formula for no more toxic metals but for non-toxic herbo-metallic compounds. Therefore, it is claimed that heavy metals are detoxified with herbal extract and excreted out from the body without any harm to body.[12] *Bhasmas* are metal preparations which are subjected to physico-chemical processing called *Samskaras* to purify, detoxify and retain the therapeutic properties. Ayurvedic experts have estimated that 35-40% of the approximately 600 medicines in the Ayurvedic formulary, intentionally contain at least one metal.[13] On the other hand, there are certain plant species, which has high affinity to absorb certain traces of metals from the soil. There are more than 60 plant species which has a natural tendency to absorb traces of metals from the soil which could be used as a natural ingredient and may be important for therapeutic efficacy. Here, trace metal might be working as an active ingredient in the plant material[14] and the possibility of presence of heavy metals in herbal, herbo-metallic and *Bhasma* are unavoidable. Hence, the aim of present study was to evaluate the effect of chronic administration of Calcury tablet (herbal), Energic-31 capsule (herbo-metallic) and *Basanta Kusumakara Rasa* (BKR) on neurobehavioral activity and oxidative stress in rat.

Materials and Methods

Experimental animals

Male wistar rats weighing 200-250 g were used in the present study. Rats were randomly divided into 14 groups with 6 rats in each group. The rats were obtained from the Central Animal House Facility of All India Institute of Medical Sciences, New Delhi and housed in the departmental animal house. The rats were group housed in polyacrylic cages (38 × 23 × 10 cm) with not more than 4 animals per cage and maintained under standard laboratory conditions with natural dark and light cycle. They were allowed free access to standard dry rat diet (Ashirwad, Punjab, India) and tap water *ad-libitum*. However, 12 h before the behavioral test, the rats were deprived of food as this is known to enhance their motivation to perform the test.

Permission of institutional animal ethics committee

The protocol of the work mentioning details of the experimental technique, justification of the use of animals, number of animals to be used, type of anesthesia, surgical procedure to be used were reviewed and approved by the Institutional Animal Ethics Committee, All India Institute of Medical Sciences, New Delhi India (497/IAEC/09).

Drugs preparation, dose and duration of treatment

Calcury tablet (herbal), Energic-31 capsule (herbo-metallic) and BKR were purchased from market (New Delhi, India) and suspended in normal saline. Mercury chloride, lead acetate, cadmium chloride, sodium arsenite were purchased from Merck (USA) and dissolved in normal saline. These three Ayurvedic formulations have been selected on the basis of preliminary study in which seventy eight drugs were analyzed for heavy metals content. These Ayurvedic formulations contained heavy metals above permissible limit. Hence, these formulations were considered for toxicological evaluation in rats.

Each tablet of Calcury contains *Saxifraga ligulata* (150 mg), *Saccharum officinarium* (75 mg), *Boerhaavia diffusa* (75 mg), *Hazarat Yahud Pishti* (37.5 mg), *Yava Kshara* (15 mg). Extracts derived from *Parmelia perlata* (150 mg), *Crataeva nurvala* (150 mg), *Tribulus terrestris* (75 mg), *Picrorrhiza kurroa* (75 mg), *Tinospora cordifolia* (75 mg) and preservatives used were sodium Methlparaben and Sodium Propylparaben. The drug manufacturer was Charak Pharmaceutical Pvt.(I) Limited (Batch no. CA177H). Weight of each tablet was 625 mg. Human dose indicated on package insert was one tablet twice a day.

Energic-31 capsule contains *Shudha Shilajita* (450 mg), *Shankha Bhasma* (10 mg), *Tribang Bhasma* (30 mg), *Shudha Kupeelu* (50 mg), *Kukkutandtwak Bhasma* (20 mg), *Muktashukti Bhasma* (10 mg), *Swarnamakshik Bhasma* (10 mg), *Shatavari* (20 mg), *Konch ke beez* (10 mg), *Asgandh* (20 mg), *Dalcheeni* (10 mg), *Nagkesar* (10 mg), *Gokhru* (10 mg), *Sonth* (10 mg), *Loh Bhasma* (10 mg), *Lodh Pathani* (10 mg), *Chhoti Ilaichi* (10 mg), *Jabitri* (10 mg), *Suranjan Meetha* (10 mg), *Bidhara* (10 mg), *Jaiphal* (10 mg), *Moosli Safed* (10 mg), *Samundra Sosh* (10 mg), *Long* (10 mg), *Babool ka Gond* (10 g), *Talamkhana* (10 mg), *Chhoti Papal* (10 mg), *Kali Mirch* (10 mg), *Safed Chandan* (10 mg), *Akarkara* (10 mg) and *Konkol Mirch* (10 mg). Weight of each capsule content was 725 mg. The drug manufacturer was Ayurved Vikas Sansthan (Batch no. 997). Human dose indicated on package was one capsule twice a day.

Basanta Kusumakara Rasa (BKR) consists of *Prawal Bhasma*, *Chandrodaya* or *Ras Sindoor*, *Moti Pishti*, *Abhrak Bhasma*, *Raupya Bhasma*, *Suvarna Bhasma*, *Shatavari*, *Adulasa Swarasa*, *Ganna*, *Kamal Ke Phool*, *Mahuti Ke Phool*, *Kadali-Kanda*, *Malati Phool*,

Chandan, and *Kasturi*. The source of composition is Siddhayoga Sangraha. The drug manufacturer was Baidyanath (Batch no. 10). And the human dose is 125 mg/day.[15]

Animal dose were calculated from human dose per day according to the method followed by Center for Drug Evaluation and Research, Food and Drug Administration (USA), 2005.[16] Three dose of each drug (Calcury tablet, Energic-31 capsule, BKR) were selected for toxicological study according to Schedule Y of Drugs and Cosmetics Acts, 2005.[17] Three doses were human equivalent Therapeutic Dose (TD), 5 times of human equivalent Therapeutic Dose (5TD) and 10 times of human equivalent Therapeutic Dose (10TD).

Animals dose for Calcury tablets were 130, 650, 1300 mg/kg, for Energic-31 capsule were 150, 750, 1500 mg/kg and for BKR were 26, 130, 260 mg/kg. All the solutions were prepared in such a way that each animal was administered solution less than 1 ml. Solutions for Calcury tablet were 50, 200, 300 mg/ml, for Energic-31 capsule were 50, 200, 400 mg/ml and for BKR were 10, 50, 100 mg/ml. All the drug solutions were administered orally to rats for 28 days. The doses and concentration for mercury chloride, lead acetate, cadmium chloride, sodium arsenite was 10 mg/kg/day and 5 mg/ml were administered orally to rat for 28 days.[18]

Experimental design

On day 1st (Baseline, pre-treatment) and on 29th day (post-treatment) neurobehavioral activity was assessed by elevated plus maze, foot fault apparatus, photoactometer, rota rod and passive avoidance apparatus. Animals were decapitated under anaesthesia after neurobehavioral activity test. Brain was removed and washed with ice-cold normal saline and stored at −70°C. Brain tissue was thawed and homogenized with 10 times (w/v) ice cold 0.1M phosphate buffer (pH-7.4). Aliquots of homogenate from rat's brain were used to determine glutathione (GSH), MDA level and heavy metal concentration.

Behavioral tests

Cognitive impairment was evaluated by using passive avoidance and elevated plus maze.[19] The motor incoordination was tested by using rota rod and photoactometer.[20] Only one animal was tested at a time.

Estimation of biochemical markers of oxidative stress

The oxidative stress markers, malondialdehyde (MDA) and reduced GSH levels were estimated in whole brain tissue of rats. The rats were anaesthetized under chloroform anesthesia to decapitate and the brains were quickly removed, cleaned by rinsing with chilled saline and stored at −70°C. The biochemical analysis was performed within 48 h.

Measurement of lipid peroxidation

Malondialdehyde (indicator of lipid peroxidation) was estimated by - Brain tissues were homogenized with 10 times (w/v) 0.1M sodium phosphate buffer (pH 7.4). The reagents acetic acid 1.5 ml (20%, v/v) pH 3.5, 1.5 ml thiobarbituric acid (0.8%, w/v) and 0.2 ml sodium dodecyl sulfate (8.1%, w/v) were added to 0.1 ml of processed tissue sample. The mixture was then kept in boiling water for 60 min. The mixture was then cooled with tap water and 5 ml of n-butanol: pyridine (15:1, v/v) and 1 ml of distilled water were added to it. Then the mixture was vortexed and centrifuged at 4000 rpm for 10 min. The organic layer was withdrawn and absorbance was measured at 532 nm using a spectrophotometer (specord 2000, Analytik Jena, Germany). The concentration of MDA was determined by the linear standard curve.[21]

Estimation of glutathione

Reduced GSH was measured by - Equal quantity of homogenate was mixed with 10% trichlororacetic acid and centrifuged to separate the proteins. To 0.1 ml of this supernatant, 2 ml of 0.3 M phosphate buffer (pH 8.4), 0.5 ml of 5'5-dithiobis (2-nitrobenzoic acid) and 0.4 ml of double distilled water were added. The mixture was vortexed and the absorbance was read at 412 nm within 15 min.[22]

Estimation of heavy metals

Lead, cadmium, mercury and arsenic levels were estimated in brain tissue by inductively coupled plasma –atomic emission spectrophotometer (ICP-AES, JY 2000-2, France). Brain tissues were digested by cold vapors digestion procedure. Metal levels were expressed in $\mu g/g$-wet tissue.[23,24]

Statistical analysis

All datas are expressed as mean ± SEM. Drugs treated groups were compared to normal control and positive control group using one way ANOVA with posthoc Tukey test. Difference with a $P < 0.05$ was accepted as statistically significant. All the statistical analyses were performed using software (SPSS, version 15).

Observations and Results

Effect of chronic administration of Ayurvedic formulations on learning and memory in rats

One trial passive avoidance

There was significant decrease in mean retention latencies of mercury, lead, cadmium and arsenic treated group as compared to normal control group ($P < 0.001$). However, there was no significant change in mean retention latencies of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group as compared to normal control group ($P > 0.05$) [Table 1].

Elevated plus maze

There was significant increase in mean retention transfer latencies of mercury, lead, cadmium and arsenic treated group as compared to normal control group ($P < 0.001$). On contrary, there was no significant change in mean transfer retention latencies of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group as compared to normal control group ($P > 0.05$) [Table 1].

Effect of chronic administration of Ayurvedic formulations on locomotor activity in rats

Photoactometer (spontaneous locomotor activity)

There was significant decrease in spontaneous locomotors activity of mercury, lead, cadmium and arsenic treated group as compared to normal control group ($P < 0.001$). However, there was no significant change in spontaneous locomotors activity of

Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group as compared to normal control group ($P > 0.05$) [Table 1].

Rota rod

There was significant decrease of retention time on rod of mercury, lead, cadmium and arsenic treated group as compared to normal control group ($P < 0.001$). However, there was no significant change in retention time on rod of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group as compared to normal control group ($P > 0.05$) [Table 1].

Effect of chronic administration of Ayurvedic formulations on oxidative stress in rats

Glutathione estimation

There was significant decrease of GSH level in brain of mercury, lead, cadmium and arsenic treated group as compared to normal control group ($P < 0.001$). However, there was no significant change in GSH level in brain of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group as compared to normal control group ($P > 0.05$) [Figure 1].

Malondialdehyde estimation

There was significant increase of MDA level in brain of mercury, lead, cadmium and arsenic treated group as compared to normal control group ($P < 0.001$). However, there was no significant change in MDA level in brain of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group as compared to normal control group ($P > 0.05$) [Figure 1].

Effect of chronic administration of Ayurvedic formulations on rat's brain heavy metals

Mercury estimation

There was significant increase of mercury concentration in brain

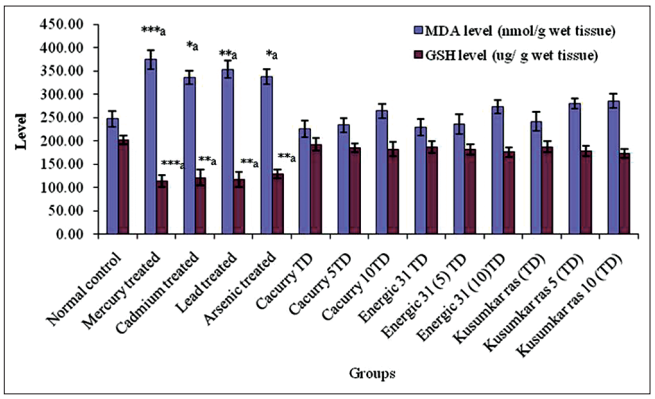

Figure 1: Effect of Ayurvedic formulations on brain malondialdehyde and glutathione levels in rats *$P<0.05$, **$P<0.01$, ***$P<0.001$, [a]As compared to normal control

Table 1: Effect of Ayurvedic formulations on learning and memory and locomotor activity

Experimental groups	Passive avoidance		Elevated plus maze		Rota rod		Photoactometer (counts/10 min)	
	ITL (s)	RTL (s)	IL (s)	RL (s)	IL (s)	RL (s)	Counts pre-treatment	Counts post-treatment
Normal control	44.52±7.57	203.33±37.22	36.3±11.12	10.5±2.3	144±19.48	156.5±10	206.75±21.06	204.25±25.6
Mercury treated	40.8±5.42	68.48±4.23***[a]	25.5±5.62	52.5±4.6***[a]	127±15.44	94±26.8*[a]	218.5±50	119.3±39.89*
Cadmium treated	36.4±7.39	119.53±6.08***[a]	26.8±7.74	41.2±6.4**[a]	144.7±19.11	109.5±22.3*[a]	238.5±34.5	123±28.79*
Lead treated	38.9±6.2	93.6±2.23**[a]	33.2±4.2	44.5±4.9**[a]	129.7±7.28	101.8±9.8*[a]	234.7±32.61	117±14*
Arsenic treated	27.4±2.57	105.02±7.49**[a]	28.2±6.38	46.0±3.2**[a]	137±16.96	107±6.4*[a]	205±18.45	132.2±5.34*
Calcury (TD)	45.95±10.51	198.62±13.22	29.33±4.9	14.33±2.74	181±21.27	145.33±15.02	216±11.37	248.17±9
Calcury (5TD)	43.25±7.34	180.1±19.98	32.33±5.4	17.33±5.45	196.17±25.81	142.5±19.71	227±14.16	228.67±13.45
Calcury (10TD)	34.12±11.65	169.68±21.1	33.0±6.4	14.67±4.13	128±31.19	146.83±24.51	246±10.76	256.33±7.7
Energic-31 (TD)	27.98±3.67	185.12±17.57	31.8±4.37	14.33±8.03	110.83±27.74	157.17±21.46	208±9.5	226.33±15.77
Energic-31 (5TD)	38.33±10.61	186.5±20.39	32.7±2.14	16.33±8.64	122.83±9.36	133.83±14.05	204.8±16.18	223.83±15.72
Energic-31 (10TD)	27.6±7.47	177.53±14.5	34.6±6.34	18.67±5.48	125.5±15.33	140±15.82	238.83±7.48	218.5±16.04
BKR (TD)	35.42±7.4	195.83±23.74	37.83±7.32	15.33±3.15	118.67±18.49	132.67±12.23	223.83±10.46	195.33±23.35
BKR (5TD)	42.17±9.32	166.57±31.7	32.83±8.37	15.17±6.24	165±9.28	168.33±12.45	257.5±9.59	192.17±17.47
BKR (10TD)	31.53±3.64	185.8±24.9	32.17±5.1	18.33±4.54	140.83±13.13	118.17±15.21	248±11.21	194.5±5.37

IL: Initial latency, RL: Retention latency, *$P<0.05$; **$P<0.01$; ***$P<0.001$; [a]As compared to normal control, ITL: Initial transfer latency, RTL: Retention transfer latency, TD: Therapeutic dose, BKR: *Basanta Kusumakara Rasa*

of mercury treated groups (203.70 ± 5.15 μg/g) as compared to normal control group (2.01 ± 0.18 μg/g) ($P < 0.001$). However, there was also significant increase of mean mercury concentration level in brain with increasing doses of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group as compared to normal control group ($P < 0.001$) but on contrary, the mercury in brain of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group was significantly less as compared to mercury treated group ($P < 0.001$) [Table 2].

Lead estimation

There was significant increase of lead concentration in brain of lead treated groups (421.90 ± 6.5 μg/g) as compared to normal control group (1.6 ± 1.5 μg/g) ($P < 0.001$). However, there was also significant increase of mean lead concentration in brain with increasing doses of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group as compared to normal control group ($P < 0.001$) but on contrary, the lead in brain of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group was significantly less as compared to lead treated group [Table 2].

Cadmium estimation

There was significant increase of cadmium concentration in brain of cadmium treated groups (289.19 ± 5.35 μg/g) as compared to normal control group (0.44 ± 0.08 μg/g) ($P < 0.001$). However, there was also significant increase of mean cadmium concentration in brain with increasing doses of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group as compared to normal control group ($P < 0.001$) but on contrary, the cadmium in brain of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group was significantly less as compared to cadmium treated group [Table 2].

Arsenic estimation

There was significant increase of arsenic concentration in brain of arsenic treated groups (88.4 ± 4.9 μg/g) as compared to normal control group (0.25 ± 0.05 μg/g) ($P < 0.001$). However, there was also significant increase of mean arsenic concentration in brain with increasing doses of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and Kusumkar ras (TD, 5TD, 10TD) treated group as compared to normal control group ($P < 0.001$) but on contrary, the arsenic in brain of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group was significantly less as compared to arsenic treated group [Table 2].

Discussion

Ayurveda is a widely practiced system in India and the used formulations are herbal, herbo-metallic and *Bhasma*. Ayurvedic text books emphasize the role of metals in proper functioning of the human body. Therefore, metals are inevitable part deliberately added and processed with herbal plants to form herbo-metallic drugs.[11,25] Ayurvedic experts have estimated that 35-40% of the approximately 600 medicines in the Ayurvedic formulary intentionally contain at least one metal.[13] *Bhasma* contains only metals which are detoxified during processing (*Shodhana*). So possibility of presence of heavy metals in herbo-metallic and *Bhasma* are unavoidable. Therefore, objective of our study was the determination of effect of chronic administration of Ayurvedic formulations on neurobehavioral activity and oxidative stress in rats.

The case reports suggests that lead content is a bigger problem with Indian HMPs and poisoning due to heavy metals have been regularly reported in the last three decades.[26-28] The mercury poisoning occurs by deposition in human cortical neuron and in a scattered group of neurons in the brain stem and cerebellum by generation of free radicals, release of intracellular calcium, lysozomal enzyme or cytoskeleton disorganization.[29] Neurological deficits due to organic mercury exposure includes encephalopathy with persistent neurological disabilities while inorganic mercury exposure produce polyneuropathy and tremor and further results decrease in visual acquity, ataxic gait and involuntary jerk movements. Most of the cognitive and emotional problems have been found in patients exposed to inorganic or organic mercury.[30]

Table 2: Effect of Ayurvedic formulations on brain heavy metal levels

Experimental group	Mercury (μg/g)	Lead (μg/g)	Cadmium (μg/g)	Arsenic (μg/g)
Normal control	2.01±0.18	1.6±1.5	0.44±0.08	0.25±0.05
Mercury treated	203.70±5.15***[a]	4.02±0.6	0.59±0.05	0.33±0.02
Cadmium treated	3.59±0.25	3.87±0.4	289.19±5.35***[a]	0.19±0.03
Lead treated	3.04±0.38	421.9±6.5***[a]	0.52±0.18	0.32±0.08
Arsenic treated	3.61±0.1	3.7±3.7	0.61±0.9	88.4±4.9***[a]
Calcury (TD)	6.08±1.31	4.9±1.4	0.48±0.06	0.38±0.16
Calcury (5TD)	6.80±1.1	5.1±1.7	0.58±0.11	0.42±0.12
Calcury (10TD)	7.61±1.14	5.9±0.3	0.61±0.14	0.46±0.04
Energic-31 (TD)	12.14±1.26	17.6±3.5	0.81±0.16	0.34±0.11
Energic-31 (5TD)	12.31±1.35	23.9±2.5	0.84±0.15	0.37±0.05
Energic-31 (10TD)	14.38±2.08	28.8±3.1	0.88±0.28	0.38±0.08
BKR (TD)	24.79±1.80	8.7±3.4	0.69±0.10	0.29±0.11
BKR (5TD)	27.04±1.30	9.7±3.6	0.74±0.22	0.34±0.07
BKR (10TD)	31.29±1.09	9.8±2.5	0.89±0.27	0.36±0.05

***$P<0.001$; [a]As compared to normal control, BKR: *Basanta Kusumakara Rasa*, TD: Therapeutic dose

On contrary, our study shows that the animals treated with Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) containing heavy metals does not cause cognition impairment and motor incoordination while positive control group in which mercury, lead, cadmium and arsenic salts were given orally to rats for 28 days caused cognition impairment and motor incoordination. The oxidative stress markers like MDA and GSH level has not been altered as compared to normal control group while there was significant decrease in GSH level and increase in MDA level in brain of positive control in which mercury, lead, cadmium and arsenic salts were given orally to rats for 28 days. We observed the higher level of heavy metal concentration in rat's brain of positive control, Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group as compared to normal control group. However, mercury, lead, cadmium and arsenic level in brain of Calcury (TD, 5TD, 10TD), Energic-31 (TD, 5TD, 10TD) and BKR (TD, 5TD, 10TD) treated group were significantly very less as compared to positive control in which mercury, lead, cadmium and arsenic salts treated group. However, no sign and symptoms, toxic manifestations were observed in these groups. Possibility of raised heavy metals level in brain could be[1] low heavy metals level exposure to rats[2] heavy metal levels were estimated immediately after 28 days administration of drugs[3] heavy metals has longer half life and[4] elimination from the body is slow. Hence, rats treated with Ayurvedic formulations showed raised heavy metal levels in brain.

The results of present study showed that herbo-metallic formulation and *Bhasma* are non-toxic even though these drugs contain metals. The reason for nontoxic nature of herbo-metallic formulation and *Bhasma* in animal could be,[1] metals in Ayurvedic formulation are not present in elemental form.[2] Physico-chemical state of the heavy metals in the form of Ayurvedic medicine is totally different from the known Physico-chemical forms of that metal.[12,13,28,31]

Heavy metal preparations (*Bhasma*) have been used in Indian System of Medicine for centuries with claimed efficacy and safety. Processed mercury shows excellent therapeutic activities in low doses without producing toxic effect in the human subjects. The toxic effects are due to impure mercury or improper use of processed mercury. The complication and toxic effects of metals has already been mentioned in Ayurveda.[8,31] However, Ayurvedic literature also mentions that metals are subjected to *Samskaras* which attributes to purification, detoxification and restoration of its therapeutic properties.[32]

Conclusion

The results of present study are coherent with the Ayurvedic literature. There were no significant changes in cognitive and motor functions and biochemical parameters of Calcury, Energic-31 and *Basanta Kusumkara Rasa* treated rats, demonstrates the safety of Ayurvedic formulations. These drugs are clinically used by a large number of populations without showing heavy metals toxicity. Hence, Calcury, Energic-31 and *Basanta Kusumkara Rasa* can be used at recommended dose and duration.

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हिन्दी सारांश

चूहों पर आयुर्वेदिक योगों के सुरक्षा मूल्यांकन

गजेन्द्र कुमार, योगेन्द्र कुमार गुप्ता

आयुर्वेदिक योगों में भारी धातुओं का प्रयोग प्रभावकारिता और सुरक्षा के साथ सदियों से होता आ रहा है । यद्यपि, धातुओं से होने वाली विषाक्तता के प्रश्न भी समय-समय पर उठाये जाते रहे हैं । वर्तमान अध्ययन का उद्देश्य calcury वटी, कैप्सूल Energic-31, बसंत कुसुमाकर रस के प्रभाव का पता लगाने का था । Male Wistar चूहों (२००-२५० ग्राम) का प्रयोग किया गया और सामान्य नियंत्रण, सकारात्मकनियंत्रण (क्लोराइड पारा, सीसा एसीटेट, कैडमियम क्लोराइड, सोडियम आर्सेनाइट, प्रत्येक १० मि.ग्राम/कि.ग्रा., २८ दिनों के लिए) और चिकित्स्य वर्ग (Calcury वटी औषध मात्रा १३०,६५०,१३०० मि.ग्रा./कि.ग्रा.; Energic – 31 कैप्सूल १५०,७५०,१५०० मि.ग्राम/कि.ग्रा; बसंत कुसुमाकर रस, १३०, २६० मि.ग्राम/कि.ग्रा.,२८ दिनों के लिए) । २९ दिन पर व्यवहार मापदंडों के प्रदर्शन के बाद चूहों के मस्तिष्क के homogenate malondialdehyde (एमडीए) और glutathione (GSH) स्तर और मस्तिष्क में भारी धातु स्तर का निर्धारण किया गया । परिणाम से ज्ञात हुआ कि सामान्य नियन्त्रण वर्ग और चिकित्स्य वर्ग में तुलनात्मक रुप से संज्ञानात्मक क्रिया, आज्ञावाही समन्वय, एमडीए और GSH स्तर में कोई महत्वपूर्ण परिवर्तन नहीं पाया गया । परन्तु, calcury वटी, Energic-31 कैप्सूल और बसंत कुसुमाकर रस चिकित्स्य वर्ग में चूहों के मस्तिष्क में भारीधातु की मात्रा का स्तर अधिक पाया गया परन्तु उसके कोई विषाक्त प्रभाव नहीं पाये गये ।