Effect of Ayurvedic mercury preparation Makaradhwaja on geriatric canine – A preliminary study

S Sinyorita¹, C K Ghosh¹, A Chakrabarti¹, B Audyy², Runa Ghosh² & P K Debnath³
¹Department of Veterinary Medicine, Ethics and Jurisprudence, West Bengal University of Animal and Fishery Sciences, Kolkata 700 037, India ²Natreon Inc. Ltd, Kolkata 700 091, India ³National Research Institute of Ayurveda for Drug Development, Kolkata 700 091, India

Received 22 June 2010; revised 30 March 2011

Makaradhwaja, an alchemical Ayurvedic mercury preparation is used as stimulant and vitalizer. Towards veterinary practices, the acceptability, tolerability and toxicity studies were undertaken in geriatric pet dogs aged more than 10 years irrespective of breed and sex for future use. Makaradhwaja (2.5 mg/kg) was used with honey once daily for 30 days. Before and after treatment, blood was collected for hematological studies as well as liver, kidney function and anti-oxidant activity. In control group, honey itself showed no appreciable change whereas, Makaradhwaja lowered neutrophil and total leucocyte count. Serum cholesterol, urea, glucose, alanine amio transferase, aspartate amino transferase, sodium, phosphorus and calcium were decreased. Haemoglobin and serum creatinine were significantly increased. There was appreciable physical, behavioral and body weight change including quality of life. The dose was used in replication of human dose (125 mg/50kg). Anti-oxidant study showed significant increase of lipid per oxidation in experimental group while the values of ABTS radical cation decolorisation assay although decreased but did not show any significant changes. Decrease of serum urea and increase of serum creatinine could not be explained on single dose response. Different dose study could only explain the optimum dose to be required in canine practices.

Keywords: Ayurvedic mercury preparation, Geriatric dog, Makaradhwaja

Makaradhwaja, a well known Ayurvedic alchemical preparation containing mercury, sulphur and gold is used in continuity through the ages as panacea. For the impending death patients it was the only resort in earlier days. How this alchemy was synthesized, who founded it, are not yet known. Makaradhwaja is known as rasayana, in Indian language the term anachronous to chemistry. The drug rasayan may be plants, minerals and metals which should have the ability to initiate the rasa (nutrient) and ayana (transportation) to attain health, longevity including rejuvenation during aging. Alchemy – Indian alchemy – Rasayana is essentially same as that of geriatrics and closely bound to the life of ascetics. In Ayurveda, it is used as general tonic and vitalizer in old age. It is also used in degenerative diseases, tuberculosis, diabetes, heart and respiratory diseases including acute fever, common cough and cold, diarrhoea, amlapitta (gastritis syndrome), chitta chanchalya (insomnia and mental anxiety) including immunomodulator and stress adaptation.

The crystals of makaradhwaja are defined as HgS and before use they are pulverized into fine powder to form nano-particulate termed as Anumakaradhwaja as described by Sir P.C.Ray. He further stated “the results of analysis of sample of mercury killed with six times its weight of sulphur along with gold the percentage of sulphur found = 13.89; the calculated amount being 13.79; not a trace of gold could be detected”. While mixing with honey, it is presumed that the atomized form saves life from death and decay. In different clinical conditions makaradhwaja is used along with honey. For better efficacy and as per requirements in different diseases fresh plant juices are mixed along with honey as anupan (vehicle). Honey is also used in rasayana against diarrhoea, eye sores, burns, wound, gastro-enteritis and peptic ulcer. The rationale for use of makaradhwaja along with honey as vehicle could not be delineated.

In veterinary practices, natural products are being usually used on replication of human applications. At the start of the present millennium, use of Ayurvedic drug in veterinary practices was emphasized for its efficacy and less toxicity.
Geriatric problems in pet animals may be recognized by some external signs, such as decreased alertness, graying of hair particularly around face and muzzle, decreasing response to visual stimuli, change in posture, increase in body fat, decreased tolerance to heat and cold, decreased strength, restlessness etc. In geriatric dogs, besides physiological disorders, behavioral, cognitive dysfunction, cardiovascular disorders, respiratory problems, nervous disorders, musculoskeletal problems, urinary problems, hepatic disorders, skin diseases, endocrine disorders, gastrointestinal diseases, immune system related problems are obvious.

In the management of aged canines care there is a dearth of geriatric remedies. On the contrary, in Ayurvedic practices makaradhwaja is being abundantly used in continuity with honey. In chronic renal failure patients and in normal volunteers makaradhwaja was found to be comparatively safe drug having no toxic component by exploiting new knowledge and new technologies.

Keeping the results of the human experiment and animal toxicity studies in view the present preliminary study has been undertaken to evaluate the acceptability and tolerability of makaradhwaja in geriatric pet canine for future use.

Materials and Methods

The geriatric dogs (12) after consent of the owner were selected randomly on the basis of their age and having no obvious clinical manifestations. Irrespective of breed and sex, 12 dogs of more than 10 years of age (12.3 ± 1.34 years) were randomly included in the study and divided into makaradhwaja treated and control honey treated group of 6 dogs each. The dogs were kept under normal nutrition and deworming even the history of free from endoparasitic as well as ectoparasitic load by regular deworming was there. After 10 days geriatric control pet canine were administered honey (Dabur), 2 ml/10 kg body weight daily for 30 days and experimental animals were administered makaradhwaja (2.5 mg/kg body weight) with honey (2 ml/10 kg body weight) once daily for 30 days (2 ml contains 25 mg makaradhwaja). Makaradhwaja was procured from J. B. Roy State Ayurvedic Medical College and Hospital, Kolkata. The dose was selected on the basis of human study (1 rati = 125 mg/50 kg body weight). All the dogs were maintained with normal diet comprising of mainly chicken, rice, papaya alternatively provided fish, egg and curd. Institutional Academic Research Committee clearance was obtained prior to experiment. Blood and serum samples were collected before and after 30 days of administration of either honey or the drug to study the anti-oxidative, hematological-biochemical, along with liver and renal function tests.

In each dog, on '0' day and 30th day 8 ml of blood was collected aseptically from recurrent tarsal and radial veins with the help of a sterilized syringe and needle. For hematological studies 3 ml of blood was transferred immediately in sterilized test tube containing dried EDTA (1 mg/ml) and the remaining 5 ml blood was transferred in a sterilized test tube for serum separation. These serum vials were transported to the laboratory in ice box. The separated serum was centrifuged in a micro centrifuge tube and preserved in deep freezeed (-20°C) for biochemical analysis.

Hematological parameters like hemoglobin (Hb) total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were determined on the same day.

The blood metabolites like serum total protein were determined by biuret method, serum albumin by using dye-binding method and globulin by the difference. The other metabolites—glucose, urea nitrogen, creatinine and cholesterol were analysed. Serum enzymes—alanine amino transferase (ALT) aspartate amino transferase (AST) and alkaline phosphatase (ALP) were also evaluated. Serum minerals like calcium were determined by cresolphthalein complexone method, phosphorus by UV end point method, sodium by Trinder method and potassium by Tetraphenyl Boron method (diagnostic reagent kit procured from Span Diagnostics Ltd, Sachin 394230, Surat, India) using spectrophotometer. The assay of malondialdehyde (MAD), produced as an index of lipid peroxidation was performed by thiobarbiturate (TBA) method and the assay of ABTS radical cation decolorisation as per Miller et al.

HPLC extraction procedure

Excitation of samples—The powdered samples were extracted with Bligh and Dyer solvent (CHCl3: MeOH: H2O 1:2:0.8, v/v/v, 10 ml × 3times), filtered and the pooled filtrate evaporated in vacuum. This was then redissolved in methanol and applied for HPLC analysis at a concentration of 5 mg/ml.

HPLC analysis—HPLC was carried out in a WATERS HPLC system with PDA detector (2996) in
isocratic mode with mobile phase consisting of acetonitrile, ortho–phosphoric acid and water (32:1:67) with a flow rate of 0.6 ml/min using a C_{18} Novapak reverse–phase column for separation. The photodiode array detector wavelength was set at 240 nm.

Data obtained were evaluated statistically by applying SPSS (version 10.0) and were expressed as mean±S E. Probability of $P<0.01$ and $<0.05$ was described as highly significant (1% level) and significant (5% level) respectively$^{21}$.

**Results**

The results of hemato-biochemical studies are shown in Tables 1 and 2. In control animals (honey) treated, the hemato-biochemical profiles revealed significantly higher percentage of neutrophil, alanine amino transferase level and lower level of total protein on 30th day as compared to 0 day. Other haematono-biochemical parameters did not show significant differences. The mean total leukocyte count, calcium and cholesterol levels were found to be significantly lower whereas, total protein and creatinine levels were significantly higher in the experimental animals after treatment with makaradhwaja.

The mean value of lipid peroxidation in experimental group of animals revealed significant increase (2.155 ± 0.058 µg/ml) as compared to healthy control group (1.947 ± 0.019µg/ml) of animals. But, the mean values of ABTS radical cation decolorisation assay did not show any significant changes.

**HPLC interpretation**—HPLC chromatogram of makaradhwaja sample is given in Fig. 1. Distinct peaks (characterized by PDA spectra) of polar proteinaceous compounds with maxima at 276 nm were observed in the sample in the $t_R$ regions 1.6–1.9 min (Fig. 2). Presence of some organo-metallic complexes were also observed in the $t_R$ regions 2.4-3.7 mins.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (gm/dl)</td>
<td>12.031 ± 0.045</td>
<td>12.014 ± 0.087</td>
</tr>
<tr>
<td>Total erythrocyte count (10^6/mm^3)</td>
<td>5.450 ± 0.174</td>
<td>5.050 ± 0.152</td>
</tr>
<tr>
<td>Total leukocyte count (10^3/mm^3)</td>
<td>8.634 ± 0.484</td>
<td>8.617 ± 0.742</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>65.166 ± 0.401</td>
<td>64.000 ± 4.328</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>4.667 ± 0.422</td>
<td>64.000 ± 4.328</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>27.500 ± 0.342</td>
<td>27.834 ± 3.637</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>2.667 ± 0.334</td>
<td>2.000 ± 0.447</td>
</tr>
</tbody>
</table>

**Table 1**—Effect of makaradhawaja on haematological parameters of dogs
[Values are mean ± SE from 6 dogs in each group]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (gm/dl)</td>
<td>6.194 ± 0.370</td>
<td>6.194 ± 0.370</td>
</tr>
<tr>
<td>Serum albumin(gm/dl)</td>
<td>2.838 ± 0.135</td>
<td>2.838 ± 0.135</td>
</tr>
<tr>
<td>Serum globulin(gm/dl)</td>
<td>3.285 ± 0.014</td>
<td>3.285 ± 0.014</td>
</tr>
<tr>
<td>Albumin:Globulin</td>
<td>0.803 ± 0.005</td>
<td>0.803 ± 0.005</td>
</tr>
<tr>
<td>Serum potassium mEq/L</td>
<td>4.120 ± 0.049</td>
<td>4.120 ± 0.049</td>
</tr>
<tr>
<td>Serum sodium mEq/Lit.</td>
<td>144.474 ± 3.274</td>
<td>144.474 ± 3.274</td>
</tr>
<tr>
<td>Serum phosphorus (mg/dl)</td>
<td>5.428 ± 0.027</td>
<td>5.428 ± 0.027</td>
</tr>
<tr>
<td>Serum calcium (mg/dl)</td>
<td>10.103 ± 0.081</td>
<td>10.103 ± 0.081</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
<td>23.826 ± 0.202</td>
<td>23.826 ± 0.202</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.445 ± 0.005</td>
<td>1.445 ± 0.005</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>236.700 ±1.101</td>
<td>236.700 ±1.101</td>
</tr>
<tr>
<td>Serum glucose (mg/dl)</td>
<td>115.618 ±1.021</td>
<td>115.618 ±1.021</td>
</tr>
<tr>
<td>Serum alanine amino transferase (IU/L)</td>
<td>48.000 ±13.226</td>
<td>48.000 ±13.226</td>
</tr>
<tr>
<td>Serum aspartate amino transferase (IU/L)</td>
<td>31.000 ± 0.695</td>
<td>31.000 ± 0.695</td>
</tr>
</tbody>
</table>

**Table 2**—Effect of Makaradhwaja on biochemical parameters in dogs
[Values are mean ± SE from 6 dogs in each group]
(Fig. 3). These complexes are characterized by absence of any specific maxima in the spectra indicating masking effects on organic molecules by metal ions present in the sample of makaradhwaja. In the \( t_R \) regions 4.0-7.0 min several peaks typical of organic compounds were observed (Fig. 4).

**Discussion**

In the present preliminary study single dose of makaradhwaja was used for 30 days extrapolating human dose to evaluate the acceptability, tolerability and toxicity in geriatric pet canine. The toxicity and acceptability studies have already been undertaken in rat, rabbit and human but not in dogs. Due to aging pet dogs mostly suffer from degenerative changes leading to physical and behavioral dysfunction affecting their quality of life. Makaradhwaja is usually used as fine powder mixed with honey. Honey itself showed some positive effect but could not be rationalized as the study was limited to one month while in Ayurveda honey is used in different ailments.

When makaradhwaja along with honey was used on aged pet dogs, there were both subjective, objective and behavioral improvements. The observable improvement was recorded in hematological picture. There was significant hypocholesterolemic effect without affecting liver function and renal efficiency. Makaradhwaja showed significant change in the oxidative stress scavenging system confirming the effects as observed in rats. In mice Makaradhwaja (25 mg/kg body weight) produced significant effect through increasing lipid per oxidation and glutathione activity. It is interesting to note that in geriatric canine there were significant changes in hemato-biochemical, cellular and renal efficiency in comparison to adult canine with honey.

After treatment with makaradhwaja in geriatric phase of pet dogs the quality of life in terms of food intake, response to command, agility and docile behavior pattern were improved. In geriatric phase stress due to adreno-cortical hyperactivity leads to hypercholesteremia. Makaradhwaja treatment decreased cholesterol level pointing towards the anti-stress activity.

In geriatric phase the basal level of serum protein, serum glucose, serum calcium, serum creatinine, serum sodium, serum phosphorus and serum cholesterol and serum urea remain at low level but the serum creatinine remained at higher side as compared to young canine (aged 2 years), may be due to aging process.

The increased serum creatinine and decreased serum urea following the administration of makaradhwaja could not be explained on the basis of present data. The increase of serum creatinine may be due to adaptation or due to aging. In geriatric dog serum urea and serum creatinine are significantly higher than the younger dogs (2-5 yrs). Serum protein level was high and kidney function deteriorated. In rats and rabbit short term treatment with makaradhwaja (high dose) led to cloudy
swelling of the kidney architecture. But in treatment for long duration (in human therapeutic dose) this change could not be confirmed. Dose response study may resolve the discrepancy. The dose employed in geriatric canine in the present study may be on higher side. Makaradhwaja has definite stimulant action on animal tissues. In rabbit, dog and in human small dose of makaradhwaja increases hemoglobin and number of red blood corpuscles including improvement in general condition and body weight.

The absorption, distribution and excretion of mercury and its compounds vary considerably on the chemical forms of the metals. Mercurial inorganic salts are less absorbed (15%) than organic once (80%)\(^6\). Presence of metals like zinc, cadmium, calcium, manganese in gut influences the absorption of inorganic mercurial. Mercurous salts are not used clinically for their toxic components. The inorganic mercurials do not pass the blood brain barrier and placenta. Administration of 1 rati (125 mg) makaradhwaja twice daily for 1 month to human volunteers and on patients of chronic renal failure did not show appreciable toxic effect in kidney function assessed on serum creatinine and serum urea level\(^7\). Ray\(^4\) had demonstrated that 25 parts of mercury by weight could only take up (combine with) 4 parts of sulphur. Usually, during preparation of makaradhwaja, mercury, gold and sulphur are mixed in the proportion 8:1:16. The excess sulphur volatilizes forming Hg\(_2\)S. The percentage of sulphur is found to be 13.89 but trace of gold could not be detected. The rationality of the use of gold and mercury in the proportion 1:8 was reported by Debnath\(^5\).

Mercury is extracted from naturally occurring cinnabar—an amalgamation of sulphur and other trace elements. For its affinity with gold, the mercury is being used in gold extraction since ancient times. The ratio of mercury and gold is always 8:1 (w/w) depending on the co-ordination number and ratio. How the ancient scientists without knowing the co-ordination number could fix this ratio of mercury and gold in the preparation of makaradhwaja is a matter for further discussion\(^6\).

On chemical analysis of makaradhwaja different microelements were detected. It is presumed that the microelements are been taken up by the metals during sodhana process\(^22\). Kumar et al.\(^28\) indicated that Siddha makaradhwaja is stoichiometrically pure Hg\(_2\)S. It does not contain other elements even traces of gold confirmed by Neutron Activation Analysis\(^28\). HPLC studies of gold bhasma revealed the presence of organic material, as organo-metallic molecule which could protect itself even on high temperature. Similarly for the first time presence of organo-metallic molecule was confirmed in makaradhwaja\(^29\). The possible source may be the nature itself or during the process of preparation as all the three raw materials were exposed to plants and animal products. The miracles of makaradhwaja remains on the unexplored organo-metallic complexation molecule which was formed during alchemical preparation either helping absorption or stimulation of energy dependant enzyme system. The present study is preliminary in nature. Detail toxicological study on different dose level in dogs is needed to clear this drug for regular safe use in veterinary practices.

Acknowledgement

Authors are thankful to Professors S. Sarkar and T. K. Mandal for biochemical and chemical analysis.

References

1. Debnath P K, Molecules of metals and minerals in Ayurveda the interior science for management of diseases, edited by Brahmananda Gupta in Paper on the ayurvedic studies The Asiatic Society, Kolkata, 2006 124


23 Datta G K & Debnath P K, Stress adaptation in Ayurveda by Immunomodulatory rasayan in Proc. Rasayan, Central Council for Research in Ayurveda and Siddha (CCRAS, Govt. of India) 2001, 60


