Indian Journal of Traditional Knowledge
Vol. 7(2), April 2008, pp. 335-340

Comparative assessment of antihyperlipidaemic action of *Tamra Bhasma*

Rajiv Kumar Rai[1]*, CB Jha[2], JPN Chansuriya[3] & KR Kohli[1],

[1]Ayurveda Research, Dabur Research Foundation, 22 Site 4, Sahibabad, Ghaziabad 201010, Uttar Pradesh; [2]Department of *Rasa Shastra*; [3]CEMS, IMS, Banaras Hindu University, Varanasi 221 005, Uttar Pradesh
E-mail: rrajiva@yahoo.com

Received 15 December 2005; Revised 22 March 2006

Metals are processed through various steps like *Shodhana*, *Marana*, *Amritikarana*, etc. to convert them into *Bhasmas*, *which* are then used as a medicine in Ayurveda for internal consumption. These processes are said to increase the bio-acceptability of the metallic preparations mentioned in Ayurveda. In the classical texts, *Marana* of metals is categorized according to various media used during the process of *Marana*, such as *Kajjali*, *Mullika*, *Gandhakadi* (mercurial compounds, herbs and sulphur containing compounds) and *Ariloha* (enemy metals), which are claimed to be superior, medium, inferior and unwarranted, respectively. *Tamra* (copper) *Bhasma* has been advocated for its therapeutic use in *Hrid Rogas.* Therefore, an attempt has been made to evaluate the relative antihyperlipidaemic efficacy of *Tamra Bhasmas* made by adopting different *Marana* procedures. The results indicate that lipid lowering capacity of *Tamra Bhasma* prepared using *Mullika* (herb) is best; whereas *Bhasma* prepared using *Kajjali* (mercurial compound) is of second grade. *Bhasma* prepared using *Gandhaka* (sulphur) is not effective for the antihyperlipidaemic activity. *Tamra Bhasma* with *Ariloha* was not tried as it has been referred not fit for human use.

Keywords: Ayurvedic drugs, *Bhasmas*, *Tamra Bhasma*

IPC Int. Cl.[8]: A61K36/00, A61P9/00, A61P9/04, A61P9/10, A61P9/12

Ayurveda is known and carried forward as ancient Indian heritage. The word Ayurveda literally means the science of life. It is time tested and trusted through thousands of the years of usage. Ayurveda in times of *Charaka* and *Sushruta* primarily used medicinal plants for the preparation of therapeutic agents. It is only the 8th century AD that Indian alchemist Nagarjuna prescribed the use of metals and minerals like – *Swarna* (gold), *Rajat* (silver), *Tamra* (copper), *Abhrak* (mica) and *Makshika* (pyrites) as medicinal agents. Due to being very effective, quick in action, smaller in dosage, palatable and having longer shelf life, the use of minerals and metals became the backbone of Ayurvedic therapeutics. The medicaments prepared with them have been rated very high in the Ayurvedic therapeutics and are in wide use for the cure of the ailing humanity since centuries for almost all diseases. The branch of Ayurveda dealing with herbo-metallic preparations is termed as *Rasa Shastra*. *Tamra* (copper) is known to Indians since *Vedic* period[1]. Its therapeutic importance was recognized during *Samhita* period but during this period, its use was very limited. Its toxic properties were also known during *Samhita* period. In medieval period, with the development of new pharmaceutical techniques, its use became popular for the treatment of gastrointestinal disorders and obesity.

Tamra is included in the group of *Lauha*/ *Dhatu* (Metals)[2]. It is classified in *Sar*/ *Sadharana Lauh* group[3]. Apart from availability of *Tamra* in native form, its different mineral and animal sources (earthworms and feathers of peacock) are also mentioned in the classics. Copper like other metals is also considered as an essential element of body for normal physiological functions[4]. Deficiency of copper leads to anemia, nervous weakness, reduced melanogenesis, weakness in connective tissue and the hypo-activity of lysyl oxidase, cytochrome C oxidase, superoxide dismutase, etc[5]. *Tamra Bhasma* is one of the primary ingredient of Ayurvedic formulations like *Prabhakar Vati*, *Hridayarnava Rasa*, etc. used for *Hrid Rogas* (cardiac disorders) [6]. Tamra is also said to be effective in hyperlipidaemias.

The metals are processed by different methods to form *Bhasmas* (ash) for therapeutic purposes. The preparation of the *Bhasma* has been divided into many steps ie *Shodhana*, *Marana*, *Amritikarana*. Copper (*Tamra*) too has to pass through all such processes of *Shodhana*, *Marana*, *Amritikarana*. These

*Corresponding author

processes make the herbo-metallic compounds bio-acceptable and safe for human use as medications. Four different methods of *Marana* are categorized on the basis of use of *Kajjali*, *Mullika*, *Gandhakadi* (mercurial compounds, herbs and sulphur containing compounds) and *Ariloha* (enemy metals) during *Marana*, which are referred as superior, medium, inferior and unwarranted, respectively[7]. These textual references need scientific exploration / evaluation of the *Bhasmas* on the therapeutic efficacy so that the Ayurvedic physicians / pharmacists can adopt the appropriate method of preparation of *Bhasma*. With this hypothesis in mind, the study was carried out on *Tamra bhasma* prepared by different methods as quoted above to evaluate the efficacy on experimental animals.

Methodology

Male albino rats weighing 150 gm were taken from the central animal house, IMS, BHU to perform the study. The animals were housed in animal room of Center of Experimental Medicine and Surgery in individual polypropylene cages under ambient conditions (controlled temperature and humidity). The rats were kept for one week before commencing the experiment for acclimatization. The animals were provided water ad lib and commercially available rat feed.

All the ingredients were separately weighed[8]. Wheat flour, milk powder and cholesterol powder were mixed well manually. Thereafter, yeast powder, sodium chloride and multi vitamins were dissolved in lukewarm water. This water was added to the mixture and dough was made. Small pellets from this dough were prepared using a manually operated palletizing machine. These pellets were baked at 100°C for 3 hrs and stored in airtight container (Table 1). The rats were divided into 5 groups each comprising of eight animals:

Group I: Normal (Control) – The rats were given commercially available standard rat feed and water ad lib.

Group II: Hyperlipidaemic (Control) – Animals were fed high fat diet (HFD) and water ad lib.

Group III: These rats were kept on HFD and *Tamra Bhasma*, prepared using *Gandhaka* (*Gandhakadi*)- Sample–T1 (10 mg/kg body weight) orally once daily.

Group IV: These rats were administered HFD and *Tamra Bhasma*, prepared using *Kajjali* (*Rasa Bhasma*)-Sample–T3 (10 mg/kg body weight) orally once daily.

Group V: The animals of this group were fed HFD and treated with *Tamra Bhasma*-prepared using *Tilparni* (*Mullika)* (Sample – T2) (10 mg/ kg body weight) orally once daily.

The daily dietary consumption of each normal and experimental rat was ascertained by giving them weighed quantity of diet for one week. Each rat was started with 9 gm/day and experiment was started when the rats consumed 15 gm/day of diet and this schedule was followed till the completion of the experiment. A suspension of weighed quantity of drug was made daily in distilled water by adding gum acacia powder and administered with the help of syringe and the rubber catheter. The dietary and treatment schedules were followed for 60 days. The body weight of each rat was recorded after 15 days interval in fasting condition.

At the end of 60 days overnight fasted rats were weighed and anaesthetized by Nembutol Sodium (30 mg/kg body weight IP). Blood samples were collected by retro-bulbar puncture, kept in centrifuge tubes and allowed to clot before centrifuging for 15 min at 1000 gm to obtain the serum. The serum was either analyzed immediately or stored at 70°C until analysis was carried out. The liver was also dissected out. One gram portion of liver of each rat was weighed after blotting on filter paper. The liver tissue was homogenized in 10 ml of isopropanol (10% w/v). The homogenate was allowed to stand for 48 hrs in stopped glass tube at 4°C. Thereafter, the homogenate was mixed well and centrifuged at 1,000 gm for 10 minutes[9]. The organic extract was removed and used for determination of total cholesterol[10]. The same method was used for getting the hepatic organic extract for the determination of triglycerides[11].

The serum lipids, i.e. total cholesterol, HDL-C and triglycerides were determined using commercially available diagnostic kits according to the protocol supplied. Serum total cholesterol was assayed according to the standard method[10]. Cholesterol reacts with hot solution of ferric per chlorate, ethyl acetate and sulphuric acid (cholesterol reagent) and gives lavender coloured complex, which is measured at 560 nm in spectrophotometer. Serum HDL was assayed according to the standard method[10]. For the assay of HDL-C, precipitating reagent supplied with the kit was added to the sample and the mixture was centrifuged at 2000 RPM for 15 minutes. The

supernatant was removed and used for the determination of HDL-C. The triglyceride level was determined using Autospan liquid gold kit from span[11]. The estimation of triglycerides involves the following enzymatic reactions –

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + Free Fatty acid}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol 3 Phosphate + ADP}$$

$$\text{Glycerol 3 Phosphate + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxy Acetone Phosphate + } H_2O_2$$

$$H_2O_2\text{+ 4AAP +p-chlorophenol} \xrightarrow{\text{POD}} \text{Quinonimine dye + } 4H_2O$$

Amino antipyrine (red colored compound), absorbance of the Quinonimine is measured at 505 nm. The serum LDL-C and VLDL-concentrations were calculated as LDL = TC – HDL– TG/5; VLDL = TG/5[12]. Values of the biochemical parameters in each group were expressed as mean ± SD. Unpaired 't' test was used to test the significance of difference between means of two groups.

Observation and results

There was marked increase in the weight of hyperlipidaemic control group compared to the normal rats. Rest other treated also showed marked increase in body weight. Body weight of rats treated with *Tamra Bhasma* (Sample–T_2) was found to have increased minimum among the treated groups (Table 2). Serum cholesterol values were found significantly increased in hyperlipidaemic rats as compared to normal rats (Fig. 1). In the treated group of animals it was found significantly decreased in the animals of group IV and V as compared to hyperlipidaemic rats (Table 3). Serum triglycerides were found significantly increased in hyperlipidaemic rats as compared to normal rats (Fig. 2). In the treated group of animals, it was found significantly decreased in the IV & V group of animals as compared to hyperlipidaemic rats (Table 4). HDL cholesterol was found significantly increased in hyperlipidaemic rats

Table 1—Composition of hyperlipidaemic diet

Contents	Hyperlipidaemic diet (gm/ 100 gm)
Wheat flour	52.6
Milk powder	23.2
Dried yeast powder	3.5
Sodium chloride	1.2
Multi vitamins	1.0
Cholesterol powder	2.5
Pork lard	16.0

Table 2—Percentage change in body weight of animals

Groups	% Increase after 15 days	% Increase after 30 days	% Increase after 45 days	% Increase after 60 days
I-Normal	23.0	32.0	35.0	39.0
II-HFD	28.0	39.0	58.0	63.0
III-*Tamra Bhasma* (Prep.with *Gandhaka*)	40.0	60.0	67.0	69.0
III-*Tamra Bhasma* (Prep.with *Kajjali*)	27.0	47.0	62.0	61.0
V-*Tamra Bhasma* (Prep.with *Tilparni*)	31.0	43.0	47.0	56.0

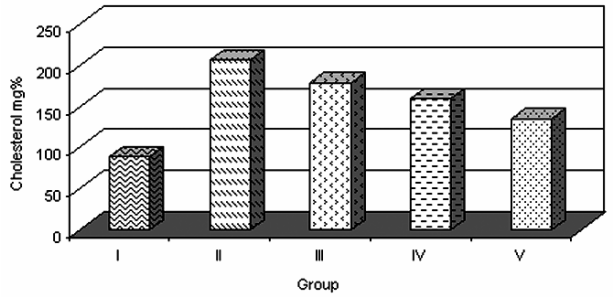

Fig. 1—Serum cholesterol in different group of animals

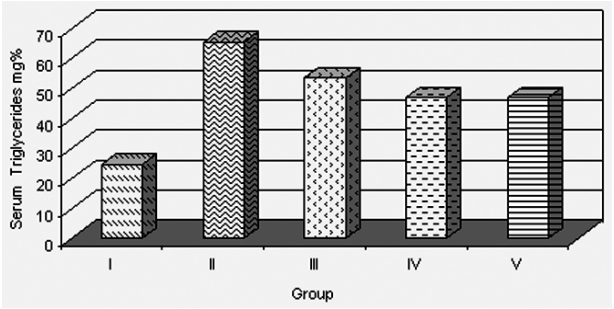

Fig. 2—Serum triglycerides in different group of animals

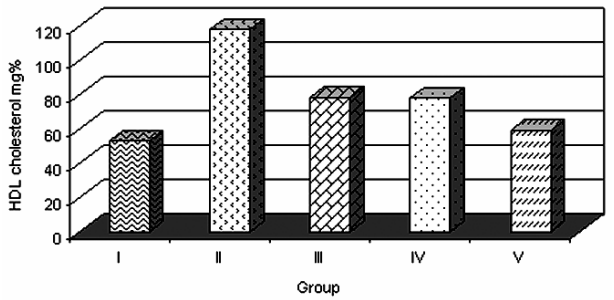

Fig. 3—HDL cholesterol in different group of animals

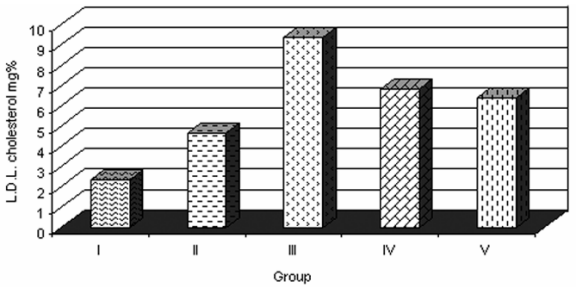

Fig. 4—LDL cholesterol in different group of animals

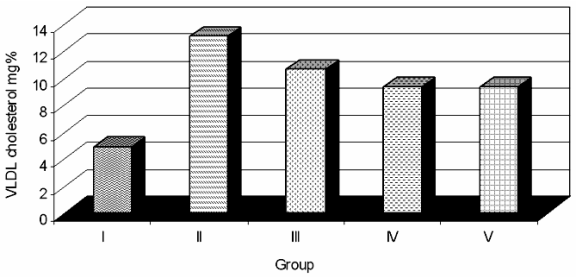

Fig. 5—VLDL cholesterol in different group of animals

Table 3—Changes in the serum cholesterol

Groups	Cholesterol Mean, SD and SE	Between the group comparison using unpaired 't' test
I (n = 8)	89.22 ± 6.89, SE = 3.08	
II (n = 8)	206.59 ± 50.52, SE = 19.09	I Vs II, t = 5.09, p<0.01
III (n = 8)	178.72 ± 42.42, SE = 14.99	II Vs III, t = 1.16, p>0.05
IV (n = 8)	159.07 ± 16.55, SE = 5.85	II Vs IV, t = 2.52, p<0.05
V (n = 8)	134.98 ± 19.74, SE = 7.46	II Vs V, t = 3.49, p<0.01

Table 4—Changes in the serum triglycerides levels

Groups	Serum triglycerides mean, SD and SE	Between the group comparison (using unpaired 't' test)
I (n = 8)	24.69 ± 6.45, SE = 2.88	
II (n = 8)	65.53 ± 11.49, SE = 2.53	I Vs II, t = 7.12, p<0.001
III (n = 8)	53.46 ± 23.49, SE = 8.31	II Vs III, t = 1.23, p>0.05
IV (n = 8)	46.93 ± 6.27, SE = 2.22	II Vs IV, t = 3.97, p<0.01
V (n = 8)	46.93 ± 6.20, SE = 2.53	II Vs V, t = 3.53, p<0.01

as compared to the normal rats (Fig. 3), whereas in treated group of animals it was found significantly increased in the IV group of animals as compared to normal rats (Table 5).

LDL cholesterol levels (Fig. 4) were found non-significant in between the group comparison, i.e. between normal and hyperlipidaemic & hyper-lipidaemic and treated group of animals (Table 6). Serum VLDL was found significantly increased in hyperlipidaemic rats as compared to normal rats (Fig. 5), whereas in treated group of animals it was found significantly decreased in IV and V group of animals as compared to hyperlipidaemic rats (Table 7).

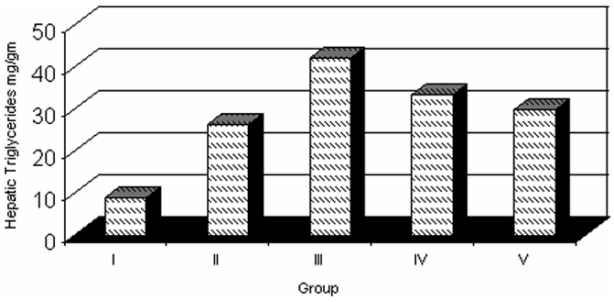

Fig. 6—Hepatic triglycerides in different group of animals

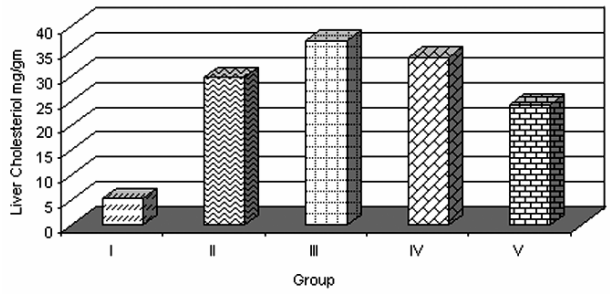

Fig. 7—Hepatic cholesterol in different group of animals

Table 5—Changes in the serum HDL cholesterol level

Groups	HDL cholesterol mean, SD and SE	Between the group comparison (using unpaired 't' test)
I (n = 8)	52.71 ± 4.96, SE = 2.22	
II (n = 8)	117.85 ± 28.26, SE = 10.68	I Vs II, t = 5.03, p<0.01
III (n = 8)	78.46 ± 30.53, SE = 10.79	I Vs III, t = 1.84, p>0.05
IV (n = 8)	78.19 ± 8.15, SE = 2.88	I Vs IV, t = 6.24, p<0.001
V (n = 8)	59.26 ± 11.90, SE = 4.50	I Vs V, t = 1.15, p>0.05

Table 6—Changes in the serum LDL cholesterol levels

Groups	LDL cholesterol mean, SD and SE	Between the group comparison (Using unpaired 't' test
I (n = 8)	2.36 ± 2.04, SE = 0.90	
II (n = 8)	4.64 ± 3.90, SE = 1.47	I Vs II, t = 1.19, p>0.05
III (n = 8)	9.36 ± 6.70, SE = 2.37	II Vs III, t = 1.63, p>0.05
IV (n = 8)	6.79 ± 2.29, SE = 0.81	II Vs IV, t = 1.33, p>0.05
V (n = 8)	6.36 ± 3.80, SE = 1.55	II Vs V, t = 0.80, p>0.05

Table 7—Changes in the serum VLDL cholesterol level

Groups	VLDL cholesterol mean, SD and SE	Between the group comparison using unpaired 't' test
I (n = 8)	4.93 ± 1.28, SE = 0.57	
II (n = 8)	13.11 ± 2.30, SE = 0.87	I Vs II, t = 7.14, p<0.001
III (n = 8)	10.69 ± 4.70, SE = 1.66	II Vs III, t = 1.23, p>0.05
IV (n = 8)	9.39 ± 1.25, SE = 0.44	II Vs IV, t = 3.97, p<0.01
V (n = 8)	9.39 ± 1.24, SE 0.51	II Vs V, t = 3.53, p<0.01

Table 8—Changes in the hepatic triglycerides level

Groups	Hepatic Triglycerides Mean, SD and SE	Between the group Comparison using unpaired 't' test
I (n = 8)	9.02 ± 1.05, SE 0.47	
II (n = 8)	26.25 ± 6.92, SE = 2.45	I Vs II, t = 5.44, p<0.001
III (n = 8)	42.06 ± 5.84, SE = 2.06	II Vs III, t = 4.94, p<0.001
IV (n = 8)	33.29 ± 4.03, SE = 1.43	II Vs IV, t = 2.48, p<0.05
V (n = 8)	29.79 ± 8.78, SE 3.32	II Vs V, t = 0.87, p>0.05

Hepatic triglyceride (Fig. 6) was found significantly increased in hyperlipidaemic rats as compared to normal rats, whereas in treated group of animals it was found significantly increased in III & IV group of animals as compared to hyperlipidaemic rats (Table 8). Hepatic cholesterol (Fig. 7) was found significantly increased in hyperlipidaemic rats as compared to normal rats, whereas in treated group animals it was found significantly increased in III group of animals as compared to hyperlipidaemic rats (Table 9).

Table 9—Changes in the hepatic cholesterol levels

Groups	Liver cholesterol mean, SD and SE	Between the group comparison (using unpaired 't' test)
I (n = 8)	5.31 ± 1.26, SE = 0.56	
II (n = 8)	29.62 ± 7.21, SE = 2.55	I Vs II, t = 7.35, p<0.001
III (n = 8)	37.11 ± 2.86, SE = 1.01	II Vs III, t = 2.74, p<.02
IV (n = 8)	33.71 ± 2.25, SE = 0.80	II Vs IV, t = 1.53, p>0.05
V (n = 8)	24.27 ± 5.07, SE = 1.92	II Vs V, t = 1.64, p>0.05

Discussion

Excessive intake of saturated fats in the diet increases the serum cholesterol levels by accelerating its biosynthesis, whereas diet containing poly unsaturated fatty acid lowers the cholesterol levels[13,14]. The experimental hyperlipidaemic diet used in present study to induce hyperlipidaemia contains large amount of saturated fats. This may explain high circulating and hepatic cholesterol levels, observed in hyperlipidaemic group of rats. Similarly, several other workers have also reported increased blood and tissue levels of cholesterol following feeding of high fat diet for varying period[8,14-16]. Such hyperlipidaemic animals have been frequently used to evaluate the hypolipidaemic activity of various herbal and synthetic drugs and therefore diet induced hyperlipidaemia is considered as better animal model for investigating antihyerlipidaemic activity because the hyperlipidaemia induced by diet is more akin to human situation.

Evaluation of *Lekhana* (weight reducing) property of *Tamra Bhasma* was done by assessing its weight reduction capacity in the experimental animals. The animals treated with *Tamra Bhasma* prepared by using *Tilparni* (gp V) showed slight reduction in body weight as compared to the animals of hyperlipidaemic control (gpII), which is not statistically significant. However, this has been found to reduce the weight more than any other group. Serum triglycerides and cholesterol significantly decreased in both the groups of animals treated with *Tamra Bhasma* prepared using *Kajjali* (gp IV) and with *Tamra Bhasma* prepared using *Tilparni* (gp V) as compared to the animals of *hyperlipidaemic* control (gp II). However, the animals treated with *Tamra Bhasma* prepared using *Gandhaka* (gp III) did not show any perceptible change in the

Table 10—Lipid lowering capacity of *Tamra Bhasma*

Tamra Bhasma prepared with	Weight reducing property	Lipid Reducing Property (antihyperlipidaemic action)					
		Serum cholesterol	Serum triglycerides	Serum VLDL	Increase in serum HDL	Hepatic cholesterol	Hepatic triglycerides
Gandhak	–	–	–	–	–	–	–
Kajjali	+	+	++	++	+	–	–
Mullika	++	++	++	++	–	–	–

levels of serum triglycerides and serum cholesterol. Serum HDL levels were significantly increased in the animals treated with *Tamra Bhasma* prepared using *Kajjali* (gp IV) as compared to the animals of normal control (gp I). Serum VLDL cholesterol levels were significantly decreased in both the groups of animals treated with *Tamra Bhasma* prepared using *Kajjali* (gp IV) and with *Tamra Bhasma* prepared using *Tilparni* (gpV) as compared to the animals of hyperlipidaemic control (gpII). Hepatic cholesterol was significantly increased in the animals treated with *Tamra Bhasma* prepared using *Gandhaka* (gp III) and in the animals of hyperlipidaimic control (gp II) as compared with the animals of normal control (gp I), whereas in rest of animals it did not show significant changes. Hepatic triglyceride levels were significantly increased in both the groups of animals treated with *Tamra Bhasma* prepared using *Gandhaka* (gp III) and using *Kajjali* (gp IV) as compared to the animals of normal control (gp I).

Conclusion

The final findings are concluded based on the therapeutic value of different methods of preparation of *Tamra Bhasma* (Table 10).

The lipid lowering capacity of *Tamra Bhasma* prepared using *Mullika* (herb) is the best, whereas *Bhasma* prepared using *Kajjali* (mercurial compound) is of second grade. *Bhasma* prepared using *Gandhaka* (sulphur) is not effective for the antihyperlipidaemic activity.

References

1 Vishva Bandhu, *Atharvaveda*, (Vishveshvaranand Vedic Research Institute, Hoshiarpur), 1960, 11/3/7.

2 Vagbhatta, *Rasa Ratna Samuchchaya*, commentary by Kulkarni, Duttatreya Ananta, (Meharchand Lakshmandas, New Delhi), 1982, 5/1.

3 Indradeo Tripathi, *Rasarnava*, (Chaukhambha Sanskrit Series, Varanasi), 1978, 7/97-98.

4 Porter JA, *Principle of chemistry*, (AS Barnes and Co New York & Chicago), 1875, 333.

5 Cunnighaum IJ, Some biochemical & physiological aspects of copper in animal nutrition, *Biochem J*, 25 (1931) 1267.

6 Das Govind, *Bhaishajya Ratnawali*, commentary by Ambikadutta Shastry, (Chaukhambha Sanskrit Series,Varanasi), 1969, 33/39-40.

7 Vagbhata, *Rasa Ratna Samuchchaya*, commentary by Kulkarni, Duttatreya Ananta, (Meharchand Lakshmandas, New Delhi), 1982, 5/14.

8 Yugarani T, Tan BHK, Teh M & Das NP, Effects of polyphenolic natural-products on the lipid profiles of rats fed high-fat diets, *Lipids*, 27-3 (1992) 181.

9 Hang A & Hostmark AT, Lipoprotein lipases, lipoproteins and tissue lipids in rats fed fish oil or coconut oil, *J Nutr*, 117 (1987) 1011.

10 Wybenga DR, Pileggi VJ, Dirstine PH & John Di Giorgio, Direct manual determination of serum Total cholesterol with a single stable reagent, *Clin Chem*, 16-12 (1970) 980.

11 P Fossati & Principe L, Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide, *Clin Chem*, 28 (1982) 2077.

12 William T Friedewald, Robert I Levy & Donald S Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, *Clin Chem*, 18-6 (1972) 499.

13 Goodnight SH, Harris WS, Conner WE & Illingworth DR, Poly-unsaturated fatty acids, hyperlipidaemia and thrombosis, *Arteriosclerosis*, 2 (1982) 87.

14 Balasubramaniam Santhirasegaram, Simons Leon A, Chang Sam & Hickie John B, Reduction in plasma cholesterol and increase in biliary cholesterol by diet rich in n-3 fatty acids in the rat, *J Lipid Res*, 26 (1985) 684.

15 Sugano N, Watanabe Shuji, Kishi A, Izume M, Ohatakaro A, Hypercholesterolemic action of chitosan with different viscosity in rats, *Lipids*, 23-3 (1988) 187.

16 Tsi D, Das NP & Taw BKK, Effect of aqueous celery (*Apium graveolens*) extract on lipid parameters of rats fed a high fat died, *Planta Med*, 61 (1995) 18.