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Research Article

AN EXPERIMENTAL STUDY OF SAMSKARA VIRUDDHA (FOOD INCOMPATIBILITY) WITH SPECIAL REFERENCE TO GHEE STORED IN BRONZE VESSEL

Abdul Sukkur M^{1*}, Shrikanth P H², Ravi Mundugaru³, Sudhakara⁴, Ravishankar B⁵

- ¹PG Scholar, Department of PG studies in Samhita, SDM College of Ayurveda, Kuthpady, Udupi, Karnataka, India
- ²Professor, Department of PG studies in Samhita, SDM college of Ayurveda, Kuthpady, Udupi, Karnataka, India
- ^{3,4}Research Officer, Department of Pharmacology and Toxicology, SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India
- ⁵Director and Professor of Experimental Medicine, SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India
- *Corresponding Author Email: drabdulsukkurm@gmail.com

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ABSTRACT

A unique principle explained by Ayurveda in relation with unsuitable food style is Viruddha Ahara (food incompatibility). Viruddha Ahara related with improper preparation of food is Samskara Viruddha. The consumption of such food that is incompatible will give rise to various ailments. The objective of this study is to evaluate the Viruddha Ahara concept of Ayurveda by experimental evidences. Wistar albino rats of either sex weighing 180±50g body weight were randomly categorized into 3 different groups, 6 rats in each group. Group 1 administered with normal rat diet and water, served as normal control. Group 2 and 3 administered with normal ghee and ghee stored in bronze vessels along with normal rat diet and water ad libitum for 60 consecutive days. On 60th day the following parameters were assed, gross behavioural, ponderal changes, haematological examination, biochemical parameters and anti oxidant level. The group 3 rats had shown hyperactivity, irritability and a significant elevation in catalase, glutathione peroxidase activity and lipid peroxidation in comparison to normal control group. The biochemical parameters revealed there is a significant reduction in the SGOT and significant elevation in the ALP, urea, creatinine and total protein and administration of both test drugs resulted in significant decrease in the total WBC count & RDW-CV in comparison to normal control group. The result suggests generation of free radicals and its oxidative damage in liver and inflammatory changes. This experiment provides a basis for the Viruddha Ahara concept mentioned in Ayurveda.

Key Words: Ayurveda, antioxidant, gross behaviour, free radicals, Viruddha Ahara.

INTRODUCTION

Ayurveda has given importance to the wholesome diet which plays a beneficial role in promotion of health and prevention of diseases. ¹A balanced diet maintains the balance among the body elements and also can be considered as a wholesome diet. The food taken as per Aharavidhi (Ayurveda dietetics) leads to proper growth and development of the body3. A unique principle explained by Ayurveda in relation with unsuitable food style is Viruddha Ahara (food incompatibility). It is stated in Samhitas that consumption of such incompatible food will give rise to various ailments.⁴ Charaka Samhita explains eighteen types of Viruddha Ahara⁵ whereas Sushruta Samhita mentioned Karma Viruddha (food incompatibility due to improper processing), Samyoga Viruddha (food incompatibility due to improper combination), Mana Viruddha (food incompatibility due to unsuitable quantity), and Rasa Viruddha (food incompatibility due to unsuitable tastes) ⁶. Viruddha concept falls under improper preparation of food under Viruddha Ahara concept 7.

Ghee is ideal for improving intelligence, memory, favouring the digestion, long life, sexual vigour and eye sight. It is also good for children and aged individuals⁸. Pure bronze has properties of Deepana (increase digestive power), Vrushya (aphrodisiac), Druk prasadana - Chakushya (good for eyes), and Sukhakara - Aarogyakara (good for proper health) ⁹. The ghee and bronze have good properties as explained but the combination of them that is the ghee when kept in bronze vessel for ten nights is considered as producing harmful or Viruddha (untoward) effects on the body. ¹⁰, ¹¹

In the present investigation an attempt is made to give experimental basis for Viruddha Ahara concept of Ayurveda.

MATERIALS AND METHOD

Experimental animals

Wistar albino rats of weighing 180 ± 50 g body weight of either sex were used in the study. The selected healthy rats were obtained from animal house attached to Pharmacology laboratory, SDM Centre for Research in Ayurveda and Allied Sciences Udupi. The experimental protocol was approved from the institutional ethical committee under the reference no. SDMCAU/IAEC-2013-14 SM-10. The animals were fed with normal rat diet and water *ad libitum* throughout the study. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. The housing provided had the following conditions: controlled lighting of 12:12h light and dark cycle, temperature of 25°C and relative humidity of approximately 50%.

Chemicals and Drugs

Test drugs - Cow's ghee (purchased from SDM Ayurveda Pharmacy Udupi) and cow's ghee stored in bronze vessel for ten nights were administered according to the body weight of the animals by oral route with the help of an oral feeding tube attached to injection syringe in morning session for 60 days. Bronze container (750 ml volume) with bronze lid was procured.

Analytical Study

The cow's ghee samples were standardized as per standard methodology of testing in Ayurvedic Pharmacopoeia of India (API). ¹²

Experimental Study

The animals are assigned into three groups of six rats each. Group 1 administered with normal rat diet and water served as control group. Group 2 was administered with cow's ghee 0.5ml/100g body weight along with normal rat diet and water. Group 3 was administered with cow's ghee stored in bronze vessel for ten nights along with normal rat diet for 60 days. On the 60th day one hour after drug administration the rats were anaesthetised and blood was collected for haematological and biochemical parameters such as SGOT, SGPT, ALP, creatinine, total protein. All the animals were sacrificed and liver was collected, weighed and stored in normal saline for preparing liver homogenate for estimating anti-oxidant activity.

Statistical Analysis

The data generated during the study were analyzed by employing one way ANOVA followed by Dennett's multiple't' test as post Hoc test for determining the level of significance of the observed effects. A 'p' value of less than 0.05 was considered as statistically significant.

RESULTS

The test samples of cow's ghee were analysed as per standard methodology of testing medicated ghee, the analytical values were in agreement with Goghrita (clarified butter, i.e. cow's ghee) of API. The samples were not rancid. The acid value indicates the presence of free fatty acids which are responsible of rancidity of the compounds; higher the free fatty acid more is the chance of samples getting rancid. Acid value of cow's ghee was 1.67 but on storing in bronze vessel the acid value has increased to 2.63. This indicates that acidity of ghee is increasing making the shelf life of the ghee short on storage in bronze vessel. Saponification value is the amount of alkali needed to saponify the fatty acids in a given quantity of oil or ghee which will depend upon number of Hydroxyl (COOH) group present. Saponification value of cow's ghee has slightly increased from 206.5 to 209.9 on storage in bronze vessel which may be due to reduction in chain length of fatty acids. Iodine value indicates the degree of unsaturation of oil. Greater the degree of unsaturation, higher will be possibility of absorption and atmospheric oxidation leading to rancidity. The more iodine number, the more unsaturated fatty acid bonds are present; unsaturated fatty acid is better absorbed than saturated fatty acids. Increase of 5.16 has been observed in iodine value on storage in bronze vessel. The ghee is getting unsaturated on storage. Detection of peroxide gives a measure of the extent to which a sample has undergone primary oxidation. Oils with a high degree of unsaturation are most susceptible to autoxidation producing peroxides as intermediates in the reaction. Autoxidation is a free radical reaction involving oxygen that leads to deterioration of fats and oils which form off-flavours and off-odours. Peroxide value, concentration of peroxide in an oil or fat, is useful for assessing the extent to which spoilage has advanced. Peroxide value has been found increasing from 1.60 to 1.99 on storage of cow's ghee in bronze vessel (Table 1).

The gross behavioural activity profile of control group rats fed with normal rat pellets and water showed no change in behavioural pattern. The behavioural change of significance of Group 2 – Cow's ghee fed rats showed hyperactivity and irritability in 2/8 rats at 1

hour after drug administration. The test drug did not produce any other behavioural changes at other time intervals to 2/8 and in other rats. The behavioural change of significance of Group 2 group – Cow's ghee kept in bronze vessel was observed of hyperactivity, irritability and rearing in 2/8 rat in 1 hour; observation of irritability and rearing in 3/8 rat in 3 hours and observation of hyperactivity and rearing in 4/8 rat in 4 hours. The test drug was not found to produce any other behavioural changes to other rats in any other time intervals

No specific changes were observed related with the food and water intake of three group rats. The following ten haematological parameters were assessed, such as haemoglobin, total WBC, RBC, Platelet, PCV, MCV, MCH, MCHC, RDW-CV, and RDW-SD. Administration of both test drugs resulted in significant decrease in the total WBC count and RDW-CV. Ghee stored in bronze vessels has shown significant decrease in the RDW-SD in comparison to normal control group (Table 2).

Administration of cow's ghee stored in bronze vessel for ten days has shown significant decrease in the serum SGOT, urea, creatinine, bilirubin direct, total protein and moderate elevation of ALP in comparison to normal control group. The cow's ghee has shown statistically non significant change in the serum SGOT, SGPT, ALP, urea and significant reduction in creatinine and bilirubin direct in comparison to normal control group (Table 3).

All the group rats have shown significant increase in the body weight in comparison to initial weight and no significant changes in the liver weight in comparison to normal control group (Table 4).

The antioxidant test result has shown the ghee stored in bronze vessels has significantly elevated catalase activity and lipid peroxidation and moderate increase in the glutathione peroxidation in comparison to normal control group. The normal cow's ghee has shown nearly normal anti oxidant activity and significant elevation in the lipid peroxidation in comparison to control group (Table 5).

DISCUSSION

The pharmacological intolerance is abnormal response of the body for the ingested food. The response develops due to sensitivity to certain foods can be correlated to *Viruddha Ahara*. The enzymatic intolerance is due to the inability to digest the food. Enzymes are proteins that catalyze the chemical reaction and they act on specific substrates. The food additives are non nutritious substances which are added to improve the taste can be understood under Samskara Viruddha (improper food processing).

The prime most factors for the maintenance of health are intake of appropriate quantity of food and properly processed food. In case of ghee if it is taken in excess quantity it hampers digestive power and produces indigestion which is a difficult condition to treat. Intake of cow's ghee kept in bronze vessel for ten nights producing early signs of toxicity is very much evident from the experimental study. Here interaction between ghee and storage vessels plays a role in food incompatibility.

Fats undergo an oxidation reaction when exposed to heat or light that produces free radicals and can trigger an inflammatory response if consumed. Because unsaturated fatty acids are lacking of hydrogen atoms and are more reactionary and volatile, they are far more susceptible for this type of damage. Fats and oils with high content of unsaturated fatty acids will become rancid quickly when subjected to any type of heat or if it stored improperly. Unfortunately most boxed jarred and frozen goods at the grocery stores have been made with cheap unsaturated oils and have oxidised during the manufacturing process.

Table 1: Analytical standards of Ghee samples

Parameters	Cow's ghee API	Cow's ghee	Cow's ghee in bronze vessel
Rancidity	-	Not oxidised	Not oxidised
Acid value	-	1.67	2.63
Saponification value	Not more than 225	206.5	209.9
Iodine value (%)	Not more than 35	21.96	27.12
Peroxide value	-	1.60	1.99

API – Ayurvedic Pharmacopoeia of India

Table 2: Effect of normal ghee & ghee stored in bronze vessel on haematological parameters

Group	Haemoglobin g/dl	WBC (10³/μL)	RBC (10 ⁶ /μL)	PCV (%)	Platelet (Lakhs/	MCV (fL)	MCH (Pg)	MCHC (g/dL)	RDW- CV (%)	RDW- SD
					mm³)					(%)
Control	14.4 ± 0.32	8200 ±	7.33 ±	39.46 ±	8.02 ±	57.65 ±	19.43 ±	36.35 ±	14.45 ±	27.81 ±
		333.67	0.21	0.82	0.39	1.08	0.38	0.13	0.65	0.46
Test I	14.73 ± 1.034	6350 ±	7.08 ±	40.66	7.23 ±	58.21 ±	20.7 ±	36.16 ±	12.46 ±	26.6 ±
		774.06*	0.46	± 2.85	0.12	0.40	0.27*	0.13	0.11**	0.57
Test II	15.71 ± 0.37	5950 ±	7.66 ±	43.05	7.23 ±	58.74 ±	20.46 ±	36.45 ±	12.43 ±	25.81 ±
		256.58*	0.12	± 1.031	0.12	1.38	0.20	0.20	0.28**	0.52*

Each value represented in Mean± SEM, *p<0.05, in comparison with control group (one way ANOVA). Test group 1 – Cow's ghee, Test group 2 – Cow's ghee stored in bronze vessels for 10 nights/days.

Table 3: Effect of normal ghee & ghee stored in bronze vessel on biochemical parameters

Group	SGOT IU/L	SGPT IU/L	ALP mg/dL	Urea mg/dL	Creatinine Mg/dL	Total protein	Bilirubin T (mg/dL)	Bilirubin D (mg/dL)
			Ü	Ü	Ö	(g/dL)	, 0	()
Control	144.5 ±	81.5 ±	437.66 ±	58.50 ±	0.71 ±	6.13 ±	0.13 ±	0.071 ±
	4.73	6.99	62.61	2.01	0.04	0.13	0.016	0.004
Test I	125.66 ±	71.16 ±	381 ±	56.33 ±	0.53 ±	7.58 ±	$0.046 \pm$	0.033 ±
	5.55	5.33	32.29	2.22	0.05*	0.24**	0.008**	0.015^*
Test II	116.66 ±	$76.83 \pm$	556.5 ±	44.66 ±	0.53 ±	7.36 ±	0.083 ±	0.021 ±
	6.66**	4.99	102.48	3.71 **	0.02*	0.34**	0.003**	0.004**

Each value represented in Mean± SEM, *p<0.05, **P<0.01 in comparison with control group (one way ANOVA). Test group 1 – Cow's ghee, Test group 2 – Cow's ghee stored in bronze vessels for 10 nights/days.

Table 4: Effect of normal ghee and ghee stored in bronze vessel on ponderal changes

Group	Body weight Initial (g)	Body weight After 28 days (g)	Liver weight (g)
Control	213 ± 5.228	243 ± 10.279**	6.13 ± 0.22
Test I	172.16 ± 3.150	$186.66 \pm 3.48^{**}$	6.28 ± 0.23
Test II	187 83 + 3 26	207 33 + 6.85**	6.37 ± 0.23

Each value represented in Mean \pm SEM, *p<0.05, **P<0.01 in comparison with control group. Test group 1 – Cow's ghee, Test group 2 – Cow's ghee stored in bronze vessels for 10 nights/days.

Table 5: Effect of normal ghee and ghee stored in bronze vessel on antioxidant system

Group	Catalase activity moles /mg protein/min.	GPA milli moles/mg protein/min. at 37°C	LP milli moles of MDA formed /g wet tissue
Control	2.30 ± 0.25	63.18 ± 6.91	4.12 ± 0.53
Test I	2.456 ± 0.28	71.13 ± 114.21	9.51 ± 0.92**
Test II	$3.79 \pm 0.345**$	92.01 ± 13.62	15.36 ± 0.28**

Each value represented in Mean± SEM, **P<0.01 in comparison with control group (one way ANOVA). Test group 1 – Cow's ghee, Test group 2 – Cow's ghee stored in bronze vessels for 10 nights/days. GPA - Glutathione peroxidase activity; LP - Lipid peroxidation



Photo 1.1 - Bronze vessel





Photo 1.3 - Bronze vessel and Cow's ghee



Photo 1.4 - Storing cow's ghee in bronze vessel



Photo 1.5 – Cow's ghee stored in bronze vessel for 10 nights/days



Photo 1. 6 - Change in bronze vessel after 10 nights/days

Figure 1: The process of storing cow's ghee in bronze vessel for 10 nights/days

In the present study the effect of freshly prepared cow's ghee and when stored in a bronze vessel for ten nights on physiology and biochemical changes in Wistar albino rats was studied.

The results of the analytical study showed increase of basic physico – chemical parameters in ghee kept in bronze vessel for ten nights in comparison with the normal ghee which is kept safely in a non corrosive container. This suggests that decomposition of ghee stored in bronze vessel started in faster pace than the normal ghee. This supports the research study which explains antagonism related with the processing (Samskara Viruddhatva) will happen when ghee is stored in bronze vessel for ten nights.

Effect on gross behaviour

The overall assessment of gross behaviour in rats showed mild hyperactivity in Test II (ghee kept in bronze vessel for ten nights) group in comparison to Group 1 (cow's ghee) and control groups. The hyperactivity is normally due to the effect on the CNS; however it is not known whether this component is involved or not. Further

evaluation of CNS activities will only give more explanations in this regard.

Effect on ponderal parameters

Body weight is a useful indicator of physical development and loss of body weight is unhealthy status of the individual. In the present study the observed weight change in body weight in both groups is found to be statistically significant in comparison within the groups. Some of the minute changes were observed such as weight gain in Group 2 is somewhat lesser than that of Group 2.

Effect on haematological parameters

The haematological result suggests that the freshly prepared cow's ghee has shown normal RBC, haemoglobin, RDWCD, packed cell volume, platelet count and the result was in comparison to normal control group except the WBC count. But there was significant increase in the mean cell volume and mean cell haemoglobin in comparison to normal control group. This shows the cow's ghee has

an influence on RBC and haemoglobulin production and hence shown the increased MCV and MCHC.

Significant decrease in the WBC count has been noted in both test groups, however the ghee stored in the bronze vessel has shown considerable decrease. The decrease in WBCs count was due to the possible reason of their getting used up while encountering a variety of inflammation injury and subsequent infections due to administration of rancid ghee. This decrease is possibly due to the failure or suppression or destruction of stem cells in the bone marrow, which leads to decrease in the number of leucocytes denoting marked decrease in the cellularity of bone marrow.

From the results projected above it can be seen that the ghee and ghee stored in bronze vessel for ten nights had some effects on the haematological parameters studied. There was non-significant effect on haemoglobin, RBC count, PCV, MCHC or platelet related parameters. The significant decrease in WBC count, RDW-CV and RDW-SD may be due to some short time effect of test drugs on these parameters. MCV showed statistical increase which indicates the immediate effect of test drugs on this parameter. By comparing the results the change in haematological parameters taken for the study was more in Group 2 (ghee kept in bronze vessel) group from that of the Group 1 (ghee).

Effect on serum biochemical parameters

The total bilirubin or conjugated bilirubin found to be elevated in liver diseases such as obstruction to biliary excretion into the duodenum, in haemolysis and defect of hepatic uptake and conjugation of bilirubin pigment. However the result from the present study has shown mild to moderate decrease in both total and direct bilirubin level in blood and decreased bilirubin in blood has less clinical importance. The phospho creatinine was present in the muscle and creatinine will form it. It is important form of energy being stored of high energy phosphate. Serum creatinine is important indicator of renal function. In the present study there is a statistically significant decrease in the serum creatinine level were observed and it indicates there is alteration in the kidney function with that of administration of test formulation. The serum total protein will rise during increased catabolism in the body. In the present study test drug has shown significant elevation in the serum total protein. This indicated there is a chance of increased protein catabolism. Estimation of serum SGOT and SGPT activity were carried out in the assessment of liver cell injury. Serum levels of above mentioned enzymes are elevated in the liver tissue damage produced by various xenobiotics. SGOT is a mitochondrial enzyme released from liver kidney, heart and skeletal muscle. Its level may rise in acute necrosis or ischemia of above mentioned organs. SGPT is a cytosolic enzyme primarily present in the liver. Hence estimation of SGOT has great value in estimating liver damage. In the present study there is a mild to moderate decrease in the SGOT in both test group and there is a significant decrease in the SGPT level in ghee stored in the bronze vessel for ten nights (days). This indicates either the test drug may severely damaged liver tissue on chronic exposure or hence there is a great decrease in the SGPT level compared to normal control group.

Serum alkaline phosphatase is produced by many tissues such as bone, liver, intestine, placenta and is excreted in the bile. Elevation in the activity of ALP thus associated diseases of liver, bone and hepatobiliary disease. In the present study there is a significant increase in the serum ALP level was observed, this might be due to the hepato biliary diseases and hence the ghee stored in the bronze vessel for ten nights (days) might cause damage to the liver tissue when administered for long duration.

Among the seven biochemical parameters studied non significant decrease was observed in SGPT activity and non significant increase was observed in alkaline phosphatase activity. This suggests the administration of test drugs has the ability to alter these parameters. There was significant increase in serum total protein level in both groups indicates the effect of ghee on this parameter irrespective of its storage conditions. The significant decrease in serum creatinine, total and direct bilirubin suggests the effect of test drugs on kidney and liver functions. By comparing the results the change in biochemical parameters considered for the study was more in Group 2 (ghee kept in bronze vessel) group from that of the Group 1(ghee).

Cellular oxidative stress is one of the important causes for development and progression of various human degenerative diseases. Several types of reactive species are generated in the body as a result of metabolic reactions in the form of free radicals or non-radicals. These species may be either oxygen derived or nitrogen derived and called pro-oxidants. They attack macromolecules including protein, DNA and lipid etc. causing cellular and tissue damage. To counter their effect, the body is endowed with another category of compounds called antioxidants.

SOD catalyses the dismutation of O₂ to produce H₂O₂. Insufficient neutralisation of hydrogen peroxide produces very aggressive hydroxyl radicals. Hence efficient system is required to scavenge the H₂O₂ effectively. They are catalase and glutathione peroxidase. In the present study the Group 2 group rats has shown elevated level of catalase and glutathione peroxidase activity. This indicates the ghee stored in the bronze vessel might have produced reactive species and generated free radical. In order to maintain cell/ tissue homeostasis there is a indirect increase in the protective anti oxidant enzymes such as catalase and glutathione peroxidase. The freshly prepared ghee has shown nearly same level as that of normal control group. Lipid peroxidation is an auto catalytic reaction involving generation of reactive oxygen species. The generated reactive species leads to cause severe oxidative damage to the living tissues. In the present study the ghee prepared freshly has shown reduced lipid peroxidation and comparable with that of normal control group. But the ghee stored in the bronze vessel for ten nights (days) has shown elevated lipid peroxidation.

Hence by considering the level of activity of catalase, glutathione peroxidase and lipid peroxidation shows the ghee stored in the bronze vessel might have undergone chemical alteration during storage period and produced moderate oxidative stress in the rats administered with the same. Hence we can conclude the ghee stored in the bronze vessel for ten nights (days) will effect on the anti oxidant level in rats.

CONCLUSION

The present finding suggests that Samskara Viruddha Ahara (food incompatibility) concept mentioned in the Ayurveda were evident from the present study and both analytical and experimental parameters support the concept. We can conclude that storing ghee in bronze vessels may impair its quality and promotes harmful effect on health. This study is an experimental evidence for an Ayurvedic concept.

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