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TOXICOLOGICAL EVALUATION OF MILK BASED RICE FLOUR BABY FOOD AS INCOMPATIBLE DIET [SAMYOGA VIRUDDHA]- AN IN VIVO STUDY

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ABSTRACT

Introduction: *Viruddha ahara* (incompatible food) is one of the novel concepts given by the science of *Ayurveda*. It includes the incompatible food which hampers the normalcy of the *Doshas* and remains harmful to the bodily tissues.Fortified rice flour is the most common first solid food given to infants. It is with milk solid or have to be reconstituted with cow milk. **Objective:** To determine sub- acute toxicity of consumption of milk–based rice flour baby food in wistar rats with special reference to *Samyoga Viruddha*. **Material and Methods:** A sub-acute study was conducted on wistar rats following repeated dose. 28 days oral toxicity study was done according to 407 OECD guidelines. Various haematological, biochemical parameters along with histopathological examination were carried out to determine toxicological implication. **Results:** Repeated administration of milk based rice flour baby food and rice flour with milk did not show statistically significant changes in haematological, biochemical parameters. Histopathological examination of liver in both the group showed granular degenerative changes of mild to moderate severity. **Conclusion:** Changes in th liver histopathology indicates sub-acute toxicity with milk-based baby rice flour baby food. It shoes that Grain flour should not be mixed with milk. It is *Samyoga viruddha* or incompatible diet.

KEYWORDS: milk base rice flour baby food, incompatible diet, *Samyoga viruddha.*

INTRODUCTION

Viruddha ahara (incompatible food) is one of the novel concepts given by the science of *Ayurveda*. It includes the incompatible food which hampers the normalcy of the *Doshas* and remains harmful to the bodily tissues.[1] Indulgence in incompatible diets causes cascading effect on *Doshas* but do not eliminate them from the body. Unwholesome food tends to cause disequilibrium with the body system and acts as a poison. Several disease entities may be caused as a result of *Viruddha ahara* including the eight *Maharogas*, genetic disturbances and even sometimes the death of the person.[2] After the first six months post-partum, breast milk becomes inadequate for the optimal growth and development of babies and rice flour based foods are often introduced to increase nutrient intake and energy level.[3] Fortified rice flour is the most common first solid food given to infants. It is with milk solid or have to be reconstituted with cow milk.[4]

But according to *Sushruta Samhita* Grain flour should not be mixed with milk. Both the ingredients become harmful to the body when mixed and consumed together.[5] Many a times these products are aggressively marketed on all the platforms. These products are also advertised as more hygienic than homemade food. Thus, a need was felt to empirically prove the efficacy of the concept of *Samyoga Viruddha* with special reference to the combination of rice flour and cow milk.

MATERIAL AND METHOD

Collection and authentication of test drug

Sample of milk - based rice flour baby food, rice and milk was collected from an authentic source. The authentication of milk - based rice flour baby food, rice flour and milk was done from well-known institute. Authentication of raw drug is mainly concerned with microscopic, macroscopic, chemical evaluations etc. After obtaining rice grains, it was ground in grinder for making it into flour. Then the product was sieved through sieve no 85.The sieved rice flour was weighed and stored in airtight container.

Experimental animals and sample size

A total of 18 animals, healthy Albino Wistar rats 21 -23 days old were used in this study. Inbred Wistar strain of rats was housed at standard environmental conditions. Body weight ranging from 50-120gm.

Acclimatization was done 7 days prior to the experimental study. Rats had free access to food and water during the acclimatization period except on previous night of the experiment i.e, overnight fasted. Animals were housed in polypropylene cages. Room temperature was maintained to 20 ± 2 ºC and relative humidity of 50-60%. Lighting was artificial with 12 hours dark/ light cycle. The cages were cleaned on a daily basis. The illumination in animal house was controlled to give approximately a sequence of 12 hr. They were fed on standard chow and provided purified water ad libitum. Animals were marked with permanent marker for proper identification on their tail.

The below table shows group of animals for the study.

Sr. no.	Group name	Dosing Schedule	No. of animals
1.	Control Group	standard diet orally for 28 days	6
2.	Test Group 1	Instant milk - based rice flour baby food along with standard diet orally for 28 days.	6
3.	Test group 2	Rice flour mixed with cow milk along with standard diet orally for 28 days.	6
			Total = 18

Experimental study design

A sub-acute study was conducted on wistar rats following repeated dose. 28 days oral toxicity study was done according to 407 OECD guidelines.[6] The study was initiated after the approval of protocol by Institutional Animal Ethics Committee (SCI/IAEC/2022-23/26).

Study Design

- Pre–clinical study / Animal Study: In-Vivo experiment.

The animals in the study were divided into 3 groups, each containing of 6 animals. The groups were named as: control group which received standard diet for 28 days, Test Group 1 which received instant milk based rice flour baby food along with standard diet orally for 28 days and Test Group 2 which received rice flour mixed with cow milk along with standard diet orally for 28 days. After 28 days of the study, body weight were observed and blood was collected from the retro-orbital plexus to estimate various parameters namely CBC, S.Bilirubin, SGOT, SGPT, S.Alkaline Phosphatase, Blood Urea, S. Creatinine, S.Cholesterol, Random Blood sugar, C-Reactive Protein, histopathology of organs were done after sacrificing the animals by euthanasia for autopsy.

Dose fixation[7]

As it is food article, its dose is difficult to determine. In infants > 6 months old, 25 gram of infant baby food is usually used to make one serving. WHO recommends 2-3 servings per day for baby > 6 months old. Average infant milk based rice flour baby food consumed by 6 months old baby is estimated to be 50 grams/ day considering wastage, spillage, leftover .Average weight of 6 months old baby is around 8 kg.

Weight of average 21 day baby rat is 100gms.[8] After using formula to fix dose in rats its came as 625 mg of infant milk based rice flour baby food to be added to 2ml of deionised water to make smooth solution.

According to American Academy of Paediatrics 6 month's old child should have 3 to 5 tablespoon (38 -63grams) of cereals in a day. Therefore amount of rice flour for 6months old child is estimated at 50 grams/day considering wastage, spillage, and leftover. After using formula to fix dose in rats its came as 625 mg of rice flour to be added to 2ml of boiled cow milk to make smooth solution.

Study Parameters

Blood sample were taken from retro orbital plexuses using capillary tubes under anaesthesia on 29th day the haematological parameters like haematocrit, haemoglobin concentration, erythrocyte count, total and deferential leucocyte count, platelet count were measured. Determination of biochemical parameters in plasma were glucose, urea, creatinine SGOT SGPT s. Bilirubin ALP and CRP. Liver, spleen, heart were organs subjected to histopathological study.

Statistical analysis

The results were expressed bas ± SD where each value represents a minimum of 6 rats [n = 6]. The data for relative organ weights, haematology and serum bio chemistry were tested using ANOVA test. The results were considered significant at $p \leq 0.05$ level.

OBSERVATION AND RESULTS

Repeated administration of test material did not shoe statistically significant variation in haematological parameters like haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, platelet count. Repeated administration of test material did not shoe statistically significant variation in biochemical parameters like glucose, urea,

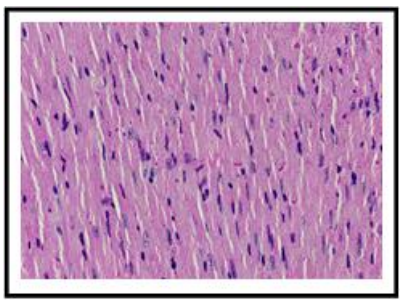
creatinine SGOT SGPT s. Bilirubin ALP and CRP. Histopathological examination of liver showed granular degenerative changes of mild to moderate severity in both the test group all other histopathological reports were normal.

Treatment	Hb gm %	RBC x 10^6/ cmm	WBC X 10^3 /cmm	PLT X 10^5/ cmm	PCV %	MCV fl	MCH pg	MCHC gm/dl	N %	E %	L %	M %
	Mean											
Normal Control	11	5.42	8.2	609.67	33.77	62.17	20.4	32.87	64.00	0.67	34.33	1.0
Baby Food	13.267	6.80	13	747.67	41.33	243.20***	19.5	32.03	61.00	1.67	37.00	0.6667
Rice Milk	13.5	6.58	15.033	761.00	41.77	63.70	20.6	32.37	70.33	1.33	27.67	0.6667
SEM	1.38	0.74	3.51	83.79	4.50	104.08	0.59	0.42	4.76	0.51	4.81	0.19
F	12.22	9.54	12.08	22.33	11.01	23.01	7.02	7.83	9.11	11.22	11.34	1.54
P Value	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P<0.001	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

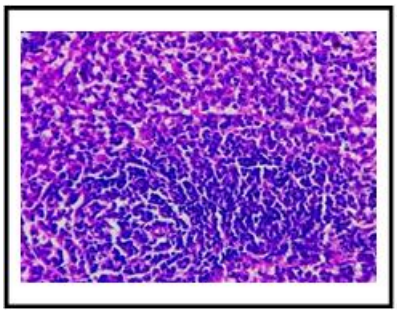
Treatment	Total Bilirubin mg/dl	AST IU/L	ALT IU/L	ALP IU/L
Normal control	0.19±0.02	133.17±5.00	157.17±7.03	68.00±3.79
Baby Food	0.18±0.02	127.50±6.69	154.67±6.22	71.67±3.67
Rice Milk	0.19±0.03	126.83±6.88	158.83±6.83	68.00±3.85
SEM	0.02	6.25	6.76	3.44
F	25.61	21.20	24.19	21.28
P Value	P>0.05	P>0.05	P>0.05	P>0.05

Treatment	Urea (mg/dl)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	CRP
	Mean				
Normal control	15.92±0.22	0.43±0.05	128.33±7.79	113.33±3.01	Not detected
Baby Food	15.82±0.29	0.39±0.04	135.33±4.08	113.83±2.71	Not detected
Rice Milk	15.63±0.18	0.42±0.05	126.50±3.45	111.67±3.14	Not detected
SEM	0.23	0.05	5.11	2.97	Not detected
F	21.23	31.15	19.2	17.22	Not detected
P Value	P>0.05	P>0.05	P>0.05	P>0.05	Not detected

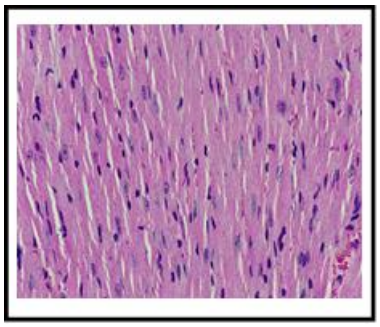
Figure: Histopathology of Heart and Spleen.



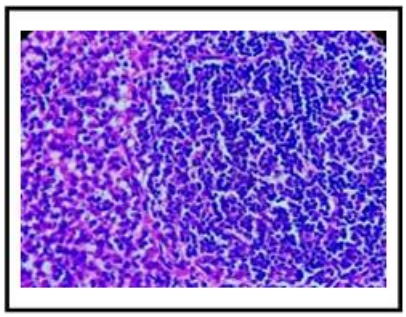
Heart Control Group



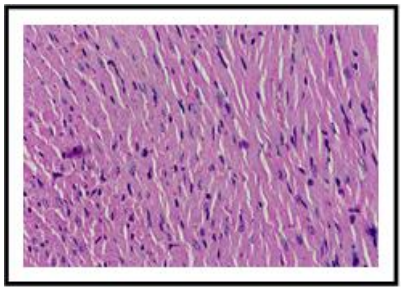
Spleen Control Group



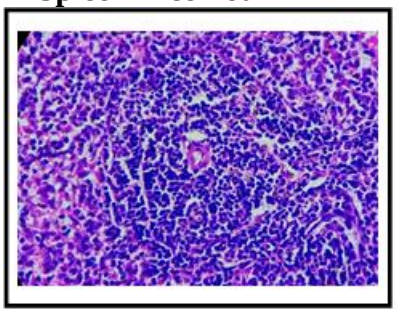
Heart Rice flour+ Milk



Spleen Rice flour +Milk

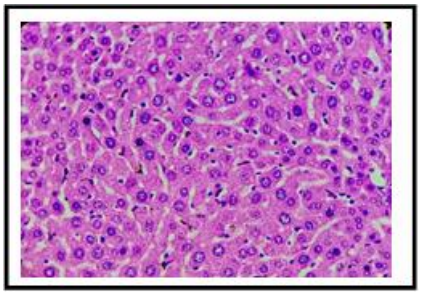


Heart Baby Food

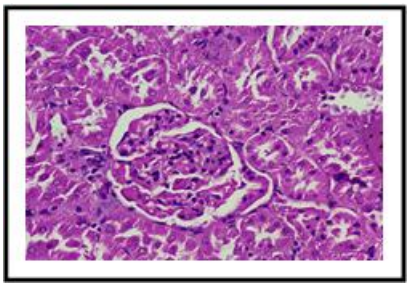


Spleen Baby Food

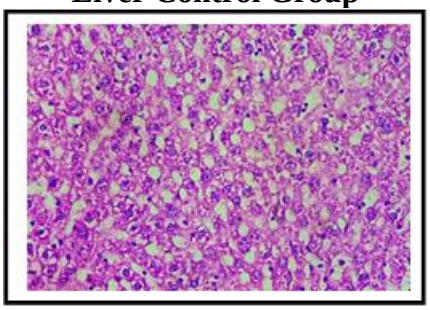
Histopathology of Liver and Kidney



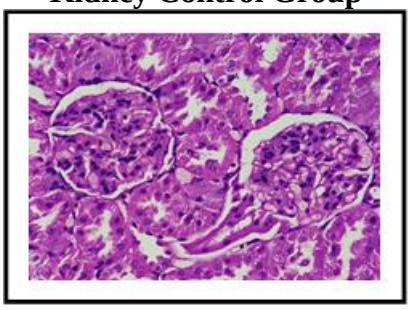
Liver Control Group



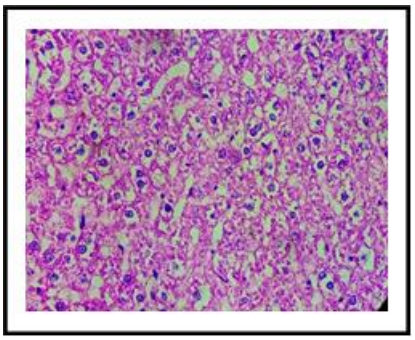
Kidney Control Group



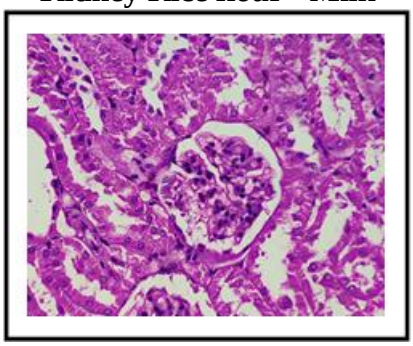
Liver Rice flour +Milk



Kidney Rice flour +Milk



Liver Baby Food



Kidney Baby Food

DISCUSSION

When two food article which when consumed separately show no harmful effects but both the food article when combined together before consuming show harmful effect on person, it is said to be *Samyoga viruddha*. Sushruta has enlisted various food articles that should not be mixed with milk while consuming.one of the food article that is forbidden is pishta i.e. grain flour. Grain flour should not be mixed with milk and consumed together. Sushruta has compared *samyoga viruddha* as *vish tulya*. It is said to be as harmful as poison.

Viruddha ahara is enlisted as one of the cause of *Raktavaha srotodushti*. As liver and spleen are stated be place of origin of *Raktavaha srotasa*, viruddha ahara is most likely to affect health of liver and spleen. *Viruddha ahara* is also one of the causes of Majjavaha *strotodushti*. As *purana* or filling the cavity of *Asthi* is one of the main functions of Majja dhatu, it can be equated with bone marrow. Bone marrow serves crucial functions in the body, primarily haematopoiesis, the process of blood cell formation. Within bone marrow, hematopoietic stem cells differentiate into various blood cell types, including red blood cells, white blood cells, and platelets. These cells are essential for oxygen transport, immune defence, and blood clotting respectively. Additionally, bone marrow houses mesenchymal stem cells, which contribute to tissue repair and regeneration.

All haematological and biochemical parameters did not show statistically significant changes in their values.

Liver tissue histopathology reveals granular degeneration of mild to moderate severity in both the test group compared to control group. Degenerative changes in liver histopathology typically indicate progressive damage to liver tissue. These alterations impair liver function, compromising metabolism, detoxification, and synthesis of essential proteins. Ultimately, untreated degenerative changes can culminate in liver fibrosis, cirrhosis, or hepatocellular carcinoma. Consumption of *Samayoga viruddha* as seen in milk- based rice flour baby food has shown mild to moderate degenerative changes in liver tissue suggestive of some toxicological implication leading to chronic liver disease consistent with *Raktavaha strotodushti*.

Kidney tissue also did not reveal any significant abnormalities. Heart and spleen tissue also did not reveal any significant abnormalities.

CONCLUSION

Test group 1 and Test group 2 showed no significant changes in the haematological parameter thereby indicating no signs of haematological toxicity. Both the test group showed no significant changes in the hepatic bio markers thereby indicating no signs of hepatic toxicity. Both the test group showed no significant changes in the creatinine and BUN levels thereby indicating no signs of renal toxicity. Both the test group showed no significant changes in the blood glucose levels, serum cholesterol and C - reactive protein levels.

Histopathological examination of kidney, heart and spleen tissue did not reveal any significant abnormalities. Histopathological examination of liver revealed mild to moderate granular degeneration tissue indicating liver toxicity for 28 days consumption in wistar rats.

Correct diet is important aspect of good health and long life. Consumption of *viruddha ahara* gives rise to various diseases. *Viruddha ahara* is implicated as one of the causes of *Raktavaha* and *Majjavaha strotodushti*. *Samayoga viruddha* is one of the types *of viruddha ahara*.

Milk –based rice flour baby food is *samayoga viruddha* food as it contains both rice flour and milk. Consumption of milk –based rice flour baby food as well as rice flour and milk by rats in both the test group led to granular degeneration of mild to moderate severity in liver.

Liver is place of origin of *Raktavaha strotas*, it implies that milk based rice flour baby food which is *samayoga viruddha* food causes *Raktavaha strotodushti*. Granular degeneration of mild to moderate severity in liver is suggestive of toxicological manifestation because of consumption of *samayoga viruddha ahara*. This study has experimentally validated the sub-acute toxicity of milk - based rice flour baby food in wistar rats with special reference to *Samyoga viruddha*.

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