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Effect of Ghee (Clarified Butter) intake on Lipid profile: A systematic review on animal experiments

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Ghee, a clarified butter commonly used in Indian cuisine for centuries. Both beneficial and harmful effects of ghee on health are found in many research works, and it creates more confusion about the intake of ghee and its effect on health. To overcome this confusion, it was decided to conduct a systematic review of experimental studies (*In vivo*) to synthesize available information on the oral intake of Ghee and its effect on lipid profile and other parameters. Twenty *in vivo* studies were identified, conducted in animals, and published between 1960 and 2020. The published research quality was assessed using ARRIVE guidelines and SYRCLE's risk of Bias tool. Three of the 20 experiments were not included in the present study as they don't have abstracts, full papers, or free access to full papers. Source of milk fat (cow/ buffalo), method of preparation (cream/curd starter or traditional/modern) and form of usage (plain/fried - oxidized) alters the composition of ghee, which affects its effect on Lipid profile (TC, TG, HDL, LDL) and other parameters. The use of ghee, along with other fatty substances, may reduce the unhealthy effects of other plain or fried oils. Quantity (5, 10, and 20% of total calories) and duration (28, 60, 90, 120, and 270 days) of ghee affect lipid profile and other parameters differently.

Keywords: Cholesterol, Ghee, Lipid profile, Systematic review, Triglycerides

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Introduction

Fatty food is an essential part of the diet, along with protein and carbohydrates. An average of 30% of total calories should come from fatty substances. Among that, an average of 10% of calories should come from saturated, poly and monounsaturated fatty acids each. Daily diet must contain less than 300 mg of dietary cholesterol along with intake of omega 3 and Omega 6 fatty acids in the proportion of 1:3 or 4[1]. In India, ghee-clarified butter is considered a superior fat, preferred for cooking and other food preparations for centuries. Ghee is commonly obtained from cow or buffalo milk. The source and method of preparation of ghee alters the composition of ghee. The major and minor constituents of cow and buffalo ghee are listed in Table 1[2].

Ghee is a major source of fat and fat-soluble Vitamins. Ghee improves growth rate and digestibility after its consumption. Ayurveda recommended Ghee not only as a food but also as a medicine. Ghee is selected as a common source of medicated fat as it has a special property to assimilate the properties of other drugs without leaving its own[3]. Ghee was recommended for longevity and protection from many diseases. Ghee also improves digestive fire (Agni) and helps in digestion and assimilation of food. Ghee improves intelligence and intellect. It strengthens the brain and nervous tissue and lubricates the connective tissue, softens the skin and internal organs[4].

In certain epidemiologic studies, the incidence of heart disease is found to be higher among the Indian immigrant population than among other ethnic populations of Western countries[5]. Consumption of Ghee along with a regular Indian dietary regimen has been attributed to an increase in the risk of cardiovascular disease in the Indian population[6]. The vulnerability of this group to CHD may be attributed to the role of dietary habits, though a definite cause was not recognized in this observation. High intake of saturated fat has been correlated with elevated plasma cholesterol levels and increased risk of CVD in humans and several animal species[7,8]. In general, Indians consume Ghee, which mainly contains 45 to 60% of saturated fatty acid of total fats and 0.15 to 0.30% of cholesterol. The American Heart Association recommends limiting the consumption of saturated fats to less than 7% of energy to reduce the risk of cardiovascular diseases[9].

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Table 1 — Major and Minor constituents of Cow and Buffalo milk Ghee[2]

Constituent	Buffalo	Cow	Constituent	Buffalo	Cow
Saponifiable Constituents			Unsaponifiable Constituents		
Triglycerides					
Short Chain (%)	45.3	37.6	Total Cholesterol (mg %)	275.0	330
Long Chain (%)	54.7	62.4	Lanosterol (mg %)	8.27	9.32
Tri-saturated (%)	40.7	39.0	Lutein (g/g)	3.1	4.2
High Melting (%)	8.7	4.9	Squalene	62.4	59.2
Partialglycerides			Carotene	00.0	7.2
Diglycerides (%)	4.5	4.3	Vitamin A	9.5	9.2
Monoglycerides (%)	0.6	0.7	Vitamin E	26.4	30.5
Phospholipids (mg %)	42.5	38.0	Ubiquinone	6.5	5
Flavor Components			Flavor Components		
Total Carbonyls	8.64	7.2	Volatile Carbonyls	0.26	0.33
Head Space Carbonyls	0.027	0.035			

As both the beneficial and harmful effect of Ghee on health is found in many research works, it creates more confusion about the intake of Ghee and its effect on health. To overcome this muddle about ill or healthy effect of Ghee, it was decided to find the effect of Ghee on lipid profile if consumed in different quantities, durations and forms (plain or fried). *In vivo* experimental studies were examined to synthesize available information on the oral intake of Ghee and its effect on lipid profile and other parameters.

Materials and Methods

Search Strategy: PubMed central (From 1960 to 2020) was searched using these search terms: Ghee + Cholesterol OR Triglyceride OR Lipid Profile, Butter + Cholesterol OR Triglyceride OR Lipid Profile**.** In addition, manual reviewing was done from the reference lists from relevant original research and reviewed articles.

Study Selection: All abstract and titles were screened by two authors to include paper addressing the effect of Ghee administration on Lipid Profile or cholesterol. We included studies that met the following inclusion criteria: 1) only Ghee was administered as source of fat; 2) animals fed with normal diet; 3) normal Healthy animals; 4) Had accessible full-text articles in English. While studies with following criteria a. single dose administration of Ghee; b. ghee along with other source of fat like other edible oil; c. medicated Ghee administration were excluded. Two thesis works was included in the present which are searched from references of other article.

Quality assessment

Study quality was evaluated for each published animal experiment by using ARRIVE (Animal Research: Reporting of in Vivo Experiments) guidelines. These guidelines on animal experiments were developed with an aim to improve the design, analysis and reporting of research. The details of the assessment of each publication as per ARRIVE guidelines are given in Table 2. All research works included in the study were coded and subjected to SYRCLE's risk of bias tool to assess the different biases (Table 3). Almost all published articles except one research work have a chance of selection bias as randomization is not followed by using a random number table or computer-generated method. All articles have detection bias as blinding of the outcome assessor is not done. Ten research works have performance bias as they have not followed randomization while housing the animals. Not a single research article is free from any type of bias.

Data collection

The details of information about used animal (Strain, Species, Sex, and Number), test drug (ghee) administration (Dose, Duration, route of administration) and effect on different parameters (Body weight, Lipid Profile) were summarized in Table 4.

Results

Twenty research articles related to Ghee intake and its effect on lipid profile were published between 1960 and 2020. The abstract or full paper of one article published in 1962 was not available online for access. Five experiments (four abstracts and one thesis work) found in the references of other article were also included in the systematic review. Out of 20 experiments, three were not included in the present study as they do not have abstract or free access to full paper. A total of 17 research works were included in the present systematic review as they fulfilled the

Table 2 — Assessment of published Animal Experiments by using ARRIVE guidelines

Research Code	01[10]	02[11]	03[12]	04[13]	05[14]	06[15]	07[16]	08[17]	09[18]	10*[19]	11[20]	12 [21]	13[22]	14[23]	15[24]	16[25]	17*[26]
Abstract	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	Y	Y	Y	NA
Introduction																	
a. Background	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
b. Objective	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Methods																	
a. Ethical statement	Y	Y	Y	N	N	N	N	N	Y	N	Y	N	N	N	Y	Y	Y
b. Study Design	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
c. Experimental procedure	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
d. Experimental animals	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
e. Housing and Husbandry	N	Y	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	N	Y	N	Y
f. Sample Size	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
g. Allocating animals to exp. Group	N	Y	N	Y	N	N	Y	N	N	Y	N	N	N	N	N	N	N
h. Experimental outcomes	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
i. Statistical methods	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y
Results																	
a. Baseline Data	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
b. Numbers analyzed	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Not Clear	Not clear	Not clear	Y	Y	Y	Y
c. Outcome and Estimation	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
d. Adverse Events	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
Discussion																	
A. Interpretation	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
B Generalisality	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Funding	Y	NM	NM	NM	Y	Y	NM	Y	Y	NM	NM	NM	Y	NM	Y	NM	NM
Strain	Rabbits	Rat	Wistar strain albino rats	Dutch-Polish rabbit	Fischer rats	Wistar rats	Albino Wistar rats	Wistar rats	*Rattus norwegicus* rats	Albino	Wistar Rats	Rabbits Oryctolagus Cuniculus	Albino SD rats	Albino Rats	Wistar Rats	Swiss Albino Mice	Witar Rats
Sex	Male	Male and female	either sex	Male	male and female	Male	Male	Male	Male	Male	Male	Male	Male	NM	Male	NM	Male
Age	NM	NM	NM	NM	NM	NM	21 days	6 week	Approx. 2 months	90 days	8 week	8 to 10 month	NM	NM	3 month	45 days	NM
Weight	1.6 kg	150 to 200 g	160±30 g	2 kg	NM	60.0 63.0 g	40–45 g	146.0± 5.12 g	145-165 g	35-40 g	200 g	---- NM	200-250 g	120-160 g	198±15 g	25±1 g	35-40 g
Number of animals	18/3 in each group	24/6 per group	30/6 in each group	40/5 in each group	5	8	8	7	8	7 in each group	5 in each group	5 in each group	5 in each group	5 in each group	8 in each group	7 in each group	6 in each group
Dose	3 g/kg	5% calorie intake (3.6 g/kg)	10 and 40 g 900 mg/kg 4140 mg/kg	NM	10 %	0.5 to 10 % of diet 0.125 to 5 g/kg of b. wt.	7 % of diet 14 g /day	2.5 and 5%	20% w/w	20% of the diet	5, 10%	2.5 g/kg b. wt.	10% diet	10% diet	31% of diet	2.5% of diet	20% of diet
Timing	Daily once	Daily once	Daily once	Daily once	------- NM	------ NM	----- Daily	------ NM	Daily once	Daily	------- NM -	Daily	------- NM	Daily	Daily	Daily	Daily
Duration	7 & 14 days	21 days	45 days	12 weeks 84 days	28 days	8 weeks	5 weeks	8 weeks	4/ 9 months	90 days	12 weeks 84 days	8 weeks 56 days	Two months 60 days	40 days	9 month	40 days	60 days
Type	Not with food	Not with food	Not with food	Not with food	Along with food	Along with food	Along with food	Along with food	Along with food	Along with food	Along with food	Along with food	Along with food	Along with food	Along with food	Along with food	Along with food

Note: Y: Yes, NM: Not Mentioned, *: full thesis is available but no research paper is available online.

Table 3 — Assessment of published Animal Experiments by using SYRCLE's risk of Bias tool.

Bias Types	Selection Bias			Performance Bias		Detection Bias		Attrition Bias	Reporting Bias	Other Bias
Research code	Allocation sequence generation and Applied	Baseline Values	Concealing of allocation	Randomized Housing of animals	Blinding of caregivers and investigators	Random Outcome assessment	Blinding of Outcome assessor	Incomplete outcome data	Selective outcome reporting	Other sources of bias
1	No	Yes	Unclear	Unclear	Unclear	Unclear	No	Yes	Yes	Yes
2	No	Yes	Unclear	Unclear	Unclear	Unclear	No	Yes	Yes	Yes
3	No	Yes	Unclear	Unclear	Unclear	Unclear	No	Unclear	Yes	Yes
4	No	Yes	Unclear	Unclear	Unclear	Unclear	No	Unclear	Yes	Yes
5	No	No	No	No	Unclear	Unclear	No	Unclear	Yes	Yes
6	No	Yes	No	Unclear	Unclear	Unclear	No	Unclear	Yes	Yes
7	No	Yes	No	Unclear	Unclear	Unclear	No	Yes	Yes	Yes
8	No	Yes	Unclear	No	Unclear	Unclear	No	Yes	Yes	Yes
9	No	Yes	Unclear	No	Unclear	Unclear	No	Yes	Yes	Yes
10	No	Yes	Unclear	Unclear	Unclear	Unclear	No	Yes	Yes	Yes
11	Yes	Yes	Unclear	No	Unclear	Unclear	No	Unclear	Yes	Yes
12	No	Yes	Unclear	Unclear	Unclear	Unclear	No	Unclear	Yes	Yes
13	No	Yes	Unclear	No	Unclear	Unclear	No	Unclear	Yes	Yes
14	No	Yes	Unclear	No	Unclear	Unclear	No	Yes	Yes	Yes
15	No	Yes	Unclear	Unclear	Unclear	Unclear	No	Yes	Yes	Yes
16	No	Yes	Unclear	Unclear	Unclear	Unclear	No	Yes	Yes	Yes
17	No	Unclear	Unclear	No	Unclear	Unclear	No	Yes	Yes	Yes

Note: Research Code no.10 and 17 are thesis.

inclusion criteria of the present study. (Fig. 1) One experiment conducted for the effect of clarified butter on memory, along with lipid profile, was also included. Thirteen experiments were conducted on rats, three experiments were conducted on rabbits, and one experiment was conducted on mice. Thirteen experiments were conducted on animals of male sex. Ghee (clarified butter) was given in 2.5, 5, 10, 20 and 31% of total daily calorie intake in 4, 4, 5, 3, and 1 experiment, respectively. In these experiments, clarified butter was administered for at least seven days and a maximum of 270 days. The research works with different specificity are summarized in Table 5.

Brief findings of all experiments

Effect of 2.5 % of fat of Total diet to animals

The 2.5% amount of ghee in the total diet of rats is approximately equal to 20 g of ghee consumed daily by adult human beings of 70 kg. This study shows a significant decrease in TC when consumed for 56 days. On the contrary, a 2.5% amount of ghee in the total diet of rabbits, which is approximately 64 g for 70 kg adult human beings, showed an increase in TC, TG, HDL, and LDL when consumed for 7 and 14 days.

Effect of 5% of the fat of total diet on animals

The 5% amount of ghee in the total diet of rats is approximately equal to 40 g of ghee consumed daily by adult human beings of 70 kg. There is a significant increase in TG, VLDL, an increase in HDL, and a decrease in body weight and LDL after 5% consumption of ghee for 21 days. There is a significant increase in body weight, TC, and TG when fat is consumed for 40 days, whereas the intake of ghee for 56 days showed a significant decrease in TC. There is an increase in body weight and a decrease in TC, TG, HDL, LDL, and cholesterol ratio when ghee prepared using cream and curd starter method is consumed for 84 days.

Effect of 10% of the fat of total diet on animals

The 10% ghee in the total diet is approximately 80 g of ghee consumed daily by an adult human being of 70 kg. A significant increase in TG and TC was observed when ghee was consumed for 28 days. There is an increase in body weight, TC, HDL and LDL after administration of ghee for 40 days. Administration of ghee in 10% for 60 days leads to a decrease in body weight and HDL, while an increase was observed in TC, TG, and LDL. Consumption of ghee prepared from cream and curd starter method for 84 days led to increased body weight, TG and HDL, while a decrease was observed in TC and LDL.

Effect of 20% of the fat of total diet on animals

The 20% amount of ghee in the total diet is approximately equal to 140-160 g of ghee consumed

Table 4 — Details of Animal experiments showing Ghee (Clarified Butter) intake and its effect on Lipid profile

Research Code	Species with strain	Sex	Number (n)	Route of administration	Dose told in Article	Dose calculated for Animal	Dose calculated for 70 kg Human adult#	Days and season	Body weight	TC	TG	HDL	LDL	VLDL	Other Parameters	Other Findings
1[10]	Rabbits	Male	3	Oral	3 g/kg/b. wt.	2.5% of total food intake	64.28 g/d	7	IN*	IN	IN	IN	IN	---	---	Dose dependent increase in TC, TG, (LDL) and decrease in RBCs count, hematocrit, glucose, and hemoglobin concentration, body weight and bile
1[10]	Rabbit	Male	3	Oral	3 g/kg/b. wt.	2.5% of total food intake	64.28 g/d	14	---	IN	IN	IN	IN	---	---	duct dilation with necrotic changes in liver histopathology with Oxidized ghee feeding. Above changes were normalized after co-administration of normal ghee.
12[21]	Rabbits	Male	5	Oral	2.5 g kg^{-1} b. wt.	2.08% of total food intake	53.57 g/d	56 Winter	DR	IN*	IN*	---	---	---	TC winter > TC summer	Higher Insulin and Glucose level in Diabetic rat fed with ghee than lucerne fed diet.
12[21]	Rabbit	Male	5	Oral	2.5 g kg^{-1} b. wt.	2.08% of total food intake	53.57 g/d	56 Summer	IN*	IN*	IN*	---	---	---	TG Summer >TG winter	2. Higher insulin level in summer season than winter in both ghee fed normal rat and diabetic rat. 3. Glucose level was high in summer than winter in diabetic rat. 4. Steady Glucose level in non-diabetic rat in both ghee and lucerne fed diet. Increase in serum insulin level in non-diabetic rat fed with fat.

Research Code	Species with strain	Sex	Number (n)	Route of administration	Dose told in Article	Dose calculated for Animal	Dose calculated for 70 kg Human adult#	Days and season	Body weight	TC	TG	HDL	LDL	VLDL	Other Parameters	Other Findings
8[17]	Wistar rats	Male	5	Oral	2.5 % of the diet	1.925 g/kg b. wt.	21.38 g/d	56	----	DR*	----	----	----	----	----	Significantly increased biliary excretion of cholesterol, bile acids, uronic acid, and phospholipids. 2. Lower levels of cholesterol esters in the serum as well as in the intestinal mucosa. 3. Not affect the 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity in the liver microsomes.
16[25]	Mice	NM	7	Oral	2.5 % of total diet	5 g/kg b. wt.	38.46 g/d	40	---	IN*	IN*	IN	---	---	---	Significant increase in TC, TG in group supplemented with 2.5 % dalda than control group. Non-significant increase in HDL in group supplemented with 2.5 % dalda than control group
2[11]	Rat	Both Sex	6	Oral	4.8 % of the diet	3.6 g.kg/ b. wt.	40g/d	21	DR	DR	IN *	IN	DR	IN *	---	Not able to conclude about beneficial effect of Cow Ghee on cognition
8[17]	Wistar rats	Male	5	Oral	5 % of the bd. wt.	3.85 g/kg bd. wt.	42.77g/d	56	---	DR*	---	---	---	---	---	---
6[15]	Wistar rats	Male	8	Oral	5 % Of the food	12.66 g/kg. b. wt.	140.66 g/d	56	IN	DR*	DR *	DR *	---	---	---	1. Heating increases COPS from 1.32 to 17.6 % and loss of phospholipids. 2. Significant increases in the levels of 7-ketocholesterol,3-keto 5-cholestene, 20-a-hydroxycholesterol, and 7-b-hydroxycholesterol after heating the ghee at 120°C for Obtaining peroxide value of approximately 26 mEq O2/kg. 3. Ghee unlikely to influence Essential Fatty Acid (EFA) status of individuals
11[20]	Wistar Rats	Male	5	Oral	5% cream of the food	3.75 g/kg b. wt.	41.66 g/d	84	IN*	DR*	DR*	IN	DR*	----	DR in Cholesterol Ratio	----
11[20]	Wistar Rats	Male	5	Oral	5% curd of the food	3.75 g/kg b. wt.	41.66 g/d	84	IN	DR*	DR*	IN	DR	----	DR in cholesterol ratio	----

(*Contd.*)

Table 4 — Details of Animal experiments showing Ghee (Clarified Butter) intake and its effect on Lipid profile (*Contd.*)

Research Code	Species with strain	Sex	Number (n)	Route of administration	Dose told in Article	Dose calculated for Animal	Dose calculated for 70 kg Human adult#	Days and season	Body weight	TC	TG	HDL	LDL	VLDL	Other Parameters	Other Findings
7[16]	Albino Wistar rats	Male	8	Oral	7% of the diet	21.77 g/kg b. wt.	241.88 g/d	35	IN	IN*	IN*	IN*	IN*	----	----	Decrease in TC, TG, LDL and HDL with soya oil than ghee. 2. Significant decrease in TC, TG, LDL and HDL after administration of cow ghee in combination with fish oil or soy oil. 3. Pronounced decrease in arachidonic acid after administration of cow ghee in combination with fish oil or soy oil
5[14]	Fischer rats	Both Sex	5	Oral	10% of the food	7.5 g/kg b. wt.	83.33 g/d	28	NM	IN	IN*	---	---	---	---	Does not have any effect on liver microsomal lipid Peroxide levels.
14[23]	Rat	NM	5	Oral	10% of total diet	7.5 g/kg b. wt.	83.33 g/d	40	IN	IN	IN	IN	IN	---	---	1.Increase in body weight and HDL in ghee group than pea nut oil group. 2. Increase in serum cholesterol, triglycerides and LDL was observed in pea nut oil group than Ghee group.
6[15]	Wistar rats	Male	8	Oral	10% Of the diet	25.33 g/kg b. wt.	281.44 g/d	56	IN	DR*	DR*	DR*	DR*	---	---	---
13[22]	Rat	Male	5	Oral	10 % of total diet	7.5 g/kg b. wt.	83.33 g/d	60	DR*	IN	IN	DR	IN	---	---	Significant increase in body weight in both fried and plain sunflower oil. 2. Normal values of TBARS acid and liver function indices in plain and fried ghee group 3. Significant increase in TC, TG, LDL and Significant reduction in HDL in plain and fried sunflower oil. 4. Maintained glutathione level and activities of CAT and SOD in plain and fried ghee than plain and fried sunflower oil.
11[20]	Wistar Rats	Male	5	Oral	10 % cream of the food	7.5 g/kg b. wt.	83.33 g/d	84	IN*	DR*	IN	IN*	DR	---	DR in cholesterol ratio	---

Research Code	Species with strain	Sex	Number (n)	Route of administration	Dose told in Article	Dose calculated for Animal	Dose calculated for 70 kg Human adult#	Days and season	Body weight	TC	TG	HDL	LDL	VLDL	Other Parameters	Other Findings
11[20]	Wistar Rats	Male	5	Oral	10% curd of the food	7.5 g/kg b. wt.	83.33 g/d	84	IN*	DR*	IN	IN*	DR	---	DR in cholesterol ratio	---
10[19]	Wister Rats	Male	7	Oral	20 % of the food	12.08 g/kg b. wt.	134.22 g/d	30	---	IN	IN*	IN	DR* (LDL+ VLDL)	---	---	a. Cow ghee opposed to buffalo ghee was more effectual in improving lipid profile and resulted in decreased deposition of cholesterol and triglycerides in aorta. b. Cow ghee opposed to soybean oil; attenuated toxicity induced by DMBA by decreasing incidence of mortality, tumor multiplicity, tumor weight, tumour volume and non-neoplastic disorders. These effects also correlated with increased accumulation of CLA and decreased lipidperoxidation in mammary tissue and increased superoxide dismutase activity in liver on cow ghee opposed to soybean oil. c. Dairy ghee opposed to soybean oil, improved immune system and antioxidative status, and cow ghee was more effectual than buffalo ghee.

(*Contd.*)

Table 4 — Details of Animal experiments showing Ghee (Clarified Butter) intake and its effect on Lipid profile (*Contd.*)

Research Code	Species with strain	Sex	Number (n)	Route of administration	Dose told in Article	Dose calculated for Animal	Dose calculated for 70 kg Human adult#	Days and season	Body weight	TC	TG	HDL	LDL	VLDL	Other Parameters	Other Findings
10[19]	Wister Rats	Male	7	Oral	20 % of the food	12.08 g/kg b. wt.	134.22 g/d	60	---	IN	IN*	IN	IN (LDL+ VLDL)	---	---	---
17[26]	Rats	Male	6	Oral	20 % of total diet	15 g/kg b. wt.	166.66 g/d	60	---	---	DR*	DR*	DR*	---	---	Dalda (Partially hydrogenated Fat) Non significant decrease in TC, but increase in LDL.
10[19]	Wister Rats	Male	7	Oral	20 % of the food	12.08 g/kg b. wt.	134.22 g/d	90	---	IN*	IN*	IN*	IN (LDL+ VLDL)	---	---	---
9[18]	*Rattusn orwegi cus* rats	Male	8	Oral	20 % of the food	15 g/kg b. wt.	166.66 g/d	120	---	IN	IN	DR	IN	---	IN in GSHPx activity after 90 days	Increase in TC, LDL and TG and decrease in HDL after administration of Corn oil. 2. GSHPx activity increased after 90 days administration of ghee.
Research Code	Species with strain	Sex	Number (n)	Route of administration	Dose told in Article	Dose calculated for Animal	Dose calculated for 70 kg Human adult#	Days and season	Body weight	TC	TG	HDL	LDL	VLDL	Other Parameters	Other Findings
9[18]	*Rattusn orwegi cus* rats	Male	8	Oral	20 % of the food	15 g/kg b. wt.	166.66 g/d	270	---	IN	DR	DR	IN	---	decline in CD and heart GSHPx activity; tissue LP products IN in GSHPx activity in liver.	. Decrease in TG after 9-month s administration of Corn oil. 2. Long period of feeding of ghee shows decline in CD and heart GSHPx activity; tissue LP products and GSHPx activity in the liver increased.
15[24]	Rat	Male	8	Oral	31 % of total diet	20.56 g/kg b. wt.	228.44 g/d	270	IN*	IN*	IN*	IN*	DR	---	DR* LDL/HDL ratio	Addition of 8 % garlic leads to mild reduction of TC. 2. Addition of 8 % garlic leads to more reduction of LDL 3. Addition of 8 % garlic leads to more reduction of TG 4. Addition of 8 % garlic leads to non-significant reduction of LDL/HDL ratio
3[12]	Wistar strain	Both Sex	6	Oral	1.2 % of the diet	0.9 g/b.wt.	10 g/d extra than diet	45	IN	DR	DR	DR	----	----	----	Insignificant prolongation of QT interval was observed after administration of ghee in dose of 40 gm.
3[12]	Wistar strain	Both Sex	6	Oral	5.52 % of the diet	4.14 g/kg b.wt.	46 g/d extra than diet	45	----	DR	DR	DR	----	----	----	

Note : IN : increase, DR: Decrease, * : Significance at 0.05 level, #:Indicate the calculated percentage of ghee of the total diet of animal or calculated Human dose by using daily intake of animal and surface area ration as per Paget and Barnes,TC- Total Cholesterol, TG- Triglycerides, HDL- High Density Lipoprotein, LDL- Low density Lipoprotein,VLDL: Very Low Density Lipoprotein, GSHPx- Glutathione peroxidase,CD- Conjugated dienes, LP- Lipid peroxidation,DMBA- Dimethylbenz(a)anthracene, CLA- conjugated linoleic acid, TBARS- Thiobarbituric Acid reactive substances, CAT- Catalase, SOD- Superoxide dismutase.

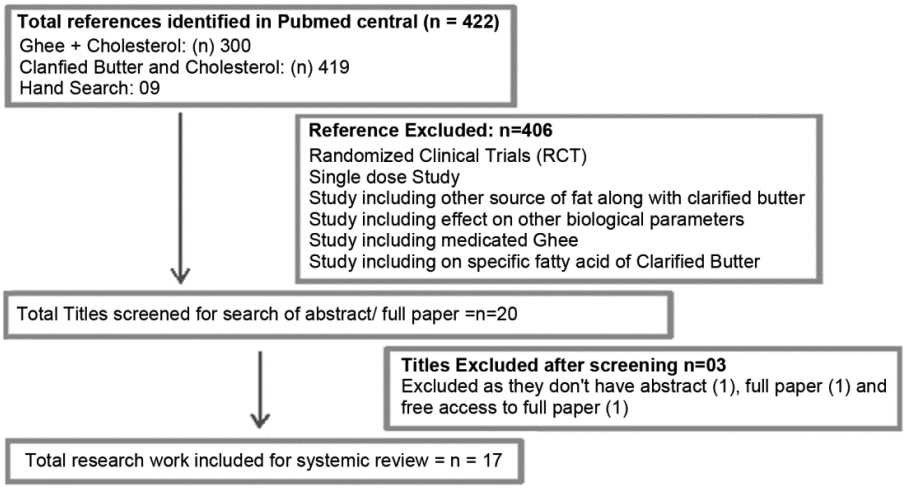

Fig. 1 — Flow diagram of the experimental studies selection process.

Table 5 — Overview of Experiments

Specificity of Experiments	Number of Research Works
Variation in Manufacturing Method of Ghee	01
Variation in assessing the effect in different seasons.	01
Use of Heated (Oxidized) Ghee along with plain ghee	02
Comparison between effect of ghee and other fat source like vegetative Oils	08
Assessment of effect of ghee for pharmacological action along with effect on lipid profile	02
To find mechanism of hypo-cholesterolemic effect of ghee	01
Effect of plain ghee on lipid Profile	01
Effect of herbs on lipid profile after consumption of high fat diet	01

daily by adult human beings of 70 kg. An increase in body weight and a significant decrease were observed in TC, TG and HDL when ghee was administered for 56 days. There is an increase in TC when ghee is administered for 30, 60, 90, 120, and 270 days, while an increase is observed in TG when ghee is administered for 30, 60, 90, and 120 days, which decreased after 270 days. There is an increase in HDL after consumption of ghee for 30, 60 and 90 days, while further consumption for 120 and 270 days decreases the HDL levels. LDL+ VLDL levels decrease when ghee is consumed for 30 to 60 days. Consumption for 90 days increases the levels of LDL+VLDL.

Effect of more than 20% of the fat of total diet on animals

There is an increase in body weight, TC, TG, HDL, and LDL when ghee is administered approximately 240 g daily to human adults for 35 days. Administration of ghee in the quantity of 280 g for 70 kg adult human for 56 days leads to an increase in body weight and a significant decrease in TC, TG, HDL, and LDL. A significant increase in body weight, TC, TG, and HDL and a decrease in LDL was observed when ghee was consumed in 31% of total food (230 g for 70 kg adult human) for 270 days.

Role of the season in the effect of ghee on lipid profile

There is a further increase in TC in winter than in summer when ghee is administered in approximately 50 g for 70 kg human for 56 days, while there was an increase in TG in summer than in winter season.

In short, the quantity and duration of ghee intake directly affect the serum level of TC, TG, LDL, and HDL. Intake of ghee up to 5% of the total diet for 84 days is helpful to regulate the lipid profile. Consumption of ghee up to 10% of total food intake for 84 days decreases TG and LDL. Further, increase in the quantity of ghee of more than 10% of total food intake for a longer duration has adverse effects on lipid profile.

Discussions

The beneficial and adverse effects of intake of clarified butter are still debatable. It depends upon many factors like the source of milk fat (cow/ buffalo), raw source-based preparation (cream/curd), method of preparation (traditional/modern), form of usage (plain/fried), dose and duration.

The buffalo ghee has a non-significant increase in body weight but significantly increases the TC, TG, VLDL and HDL when consumed for 90 days than cow's ghee[19]. Buffalo milk contains more esterified

cholesterol and less free cholesterol than cow's ghee. Milk fat is a source of conjugated linoleic acid that has anti-carcinogenic, anti-atherogenic properties[27].

According to Ganguly NC, milk fat is a good source of oleic acid, which can protect low-density lipoproteins (LDL) from oxidation and initiation of atherosclerosis[15,28]. Milk fat is also a source of short-chain fatty acids, which play an important role in maintaining mucosal integrity[29]. The short-chain and medium-chain fatty acids of milk fat are more readily absorbed, digested, and oxidized by carnitine-independent pathway without hindering the formation of chylomicrons[28,30]. As per Vinod Kansal, cow ghee decreased the expression of genes responsible for cell proliferation and raised regulated genes responsible for cell apoptosis[31,32].

Ghee prepared from curd is better than prepared from cream when given in 5% for 84 days as it lowers total cholesterol, LDL, VLDL, triglyceride levels, cholesterol ratio and increases HDL levels. Even though 10% cream-based ghee increases the triglyceride, it does not have a significant effect on liver microsomal lipid peroxidation and liver microsomal lipid peroxide levels[24]. The Ghee prepared by the traditional curd starter method than the industrial cream method leads to an increase in the presence of docosahexenoic acid (DHA) and conjugated linoleic acid (CLA), which are responsible for health benefits. DHA contributes to reduced risk of diseases like heart attack, cancer, insulin resistance, arthritis, and ADHD[33].

Administration of ghee in large quantities (20%) for 120 days significantly increases TC, LDL and TG and decreases HDL levels. However, intake for 270 days decreases the TG[22].

The plain or fried ghee (used for frying potato 5 min and reused for 4 times), in a dose of 10% of total daily calorie intake, when administered for 60 days, reduces the weight, oxidative stress, and has protective hepatic effects as compared with plain or fried sunflower oil[26]. However, ghee fried for nearly 9 hours at 160°C, in a dose of 20% of total daily calorie intake, when administered for 14 days, reduces body weight and enhances liver weight, TC, TG, and LDL[10]. The increase in liver weight is due to fat deposition in the liver. The changes that occurred in the case of fried ghee were due to the formation of peroxide value (PV), free fatty acids (FFA), thiobarbituric acid reactive substances (TBARS) and reduction on radical scavenging assay (RSA). Heating causes loss of phospholipids and oxidation of cholesterol to form cholesterol oxidative products (COPS), resulting in increased TC, TG and LDL. Heating of ghee increases COPS from 2.14 to 28.5 mg/100g. COPS are considered to be highly atherogenic in nature. Cooking leads to oxidation of triglycerides, cholesterol, and many fatty acids which may cause mutagenic and proartherosclerotic effects. According to Zeb and Islam Uddin, there is no significant increase in TC or TG when normal ghee is administered along with oxidized ghee. However, intake of normal ghee along with oxidized ghee increases the lipid profile but also prevents the bile duct dilation and necrotic changes in the liver. In short, the use of plain ghee, along with oxidized ghee, neutralizes the toxic effects of oxidized ghee. Duration of heating and temperature used during frying of ghee may affect the byproducts of oxidation, which directly affects body weight, liver weight and lipid profile[10].

Trans-fatty acid (TFA) forms in the ghee during the frying process, increasing the levels of TC. An increase in low-density lipoprotein (LDL) and a decrease in high-density lipoprotein (HDL) is due to the intake of TFA, which is usually related to the risk of coronary diseases. Using plain and fried ghee decreases oxidative stress, decreasing the glutathione level and activities of CAT and SOD[26].

The administration of ghee in 5 and 10% for 56 days can reduce the TC. Even ghee in 16% dose causes a reduction in TC after 90 days than 60 days. The decrease in TC is due to an increase in excretion of cholesterol through bile. The excretion of total bile acids is also increased by 30 to 86% when the ghee level was increased from 2.5% to 5% in the diet[17]. Cholesterol and its metabolites are excreted mainly through the bile. The ghee intake does not influence the activity of HMG-Co-A reductase in the liver, which enhances the cholesterol synthesis in the liver. Many studies also indicated that saturated fats may enhance bile flow and increase the excretion of bile components. The bile acid formation is regulated by cholesterol 7 alpha hydrolase enzyme (CYP7A1), which is the rate-limiting enzyme. Ghee had higher levels of mRNA transcripts for CYP7A1. This indicates that dietary ghee enhanced the clearance pathway and stimulated the efflux pathway, thereby exhibiting a hypocholesterolemic effect[26]. The increase in cholesterol after administration of fried ghee is due to an increase in cholesterol esterification

process. An important constituent of serum lipoproteins such as Cholesterol esters are involved in atherogenesis. Formation of cholesterol ester in the serum and intestinal mucosa is decreased due to (1) reduction in the readiness of cholesterol as an acceptor, (2) decrease in the availability of fatty acids such as 16:0, 18:2, or 20:4, which normally are utilized for esterification of cholesterol, or (3) the presence of oxysterols, which are poor substrates for the esterification reaction because of the presence of the hindering hydroxy, keto and epoxy groups. The plain ghee was rich in saturated fatty acid (C16:0 and C18:0) and oleic acid, which gets altered after frying[17].

There was a significant increase in serum levels of oleic acid and a significant decrease in levels of linoleic acid and arachidonic acid after intake of ghee. In the rabbit model, a decrease in serum LDL and atherogenesis is associated with conjugated linoleic acid, which is present in the ghee. Increased serum oleic acid levels may enable LDL to resist oxidation, which may prevent plaque formation. The same was also quoted by Sharma *et al*. as theories about the hypocholestermic effect of ghee[14]. A decrease in levels of arachidonic acid metabolites such as thromboxane and prostaglandin and decreased secretion of leukotrienes is also beneficial in preventing cardiovascular diseases[34,35].

When ghee is used along with other hypercholesterolemia diets, it has a non-significant effect on an increase in TC, TG, and LDL but significantly increases HDL. Fatty streak formation in left coronary arteries is significantly lower in rats fed with a hypercholesterolemic diet along with the ghee group than in a hypercholesterolemic diet with a hydrogenated oil group[13]. Intake of ghee leads to steady glucose levels and an increase in serum insulin levels in non-diabetic rat[21]. There is a significant decrease in TC, TG, LDL and HDL after administration of cow ghee in combination with fish oil or soya oil. The linoleic acid in the soya oil can act as a hypocholesterolemic agent, while n-3 polyunsaturated fatty acid in fish oil reduces plasma triglyceride by inhibiting hepatic secretion. A pronounced decrease was observed in arachidonic acid after administration of cow ghee in combination with fish or soya oil. Therefore intake of Ghee, fish oil, and Soya oil may have a favourable effect on lipid profile and other artherogenic parameters[16].

The season in which ghee is administered also affects the TG and TC. TC was higher in winter than summer, and TG was higher in summer than winter, even though the dose of ghee was kept constant. The proper explanation is not found for such variation, but a change in the relative plasma volume may help to explain seasonal variation[21].

The excess amount of ghee, along with other normal diets containing a normal percentage of fat from other sources for a longer duration (45 days), may decrease the serum TC, TG and LDL and increase HDL[12]. Kumar *et al.* showed that the use of plain ghee along with oxidized ghee helps to decrease the ill effects of oxidized ghee on lipid storage in the liver on histology[17].

Conclusion

Cow's ghee is more effective in controlling lipid profile than buffalo's ghee. Ghee prepared from curd and by using the traditional method (curd starter method) has good control over lipid profile and is healthier due to the presence of docosahexenoic acid (DHA) and conjugated linoleic acid (CLA). Ghee heated for a longer duration and at higher temperatures not only disturbs the lipid profile but is also unhealthy due to the production of Thiobarbituric Acid reactive substances, cholesterol oxidative products, and trans-fatty acid, but plain Ghee neutralizes the ill effects of other fatty substances like soybean oil. Administration of ghee in large quantities for a longer duration may increase total cholesterol and help regulate triglyceride levels by some regulatory method. There is a need to work on finding the threshold level of triglycerides, the duration of ghee intake, and the body's regulatory mechanism to control triglyceride levels. Intake of ghee also produces good effects like anti-carcinogenic, anti-atherogenic, immunomodulation and antioxidant effects, along with one of the fat sources required to generate calories.

Conflict of interest

The authors declare that there is no conflict of interest.

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