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Effects of commercial non-dairy tea whitener consumption in comparison to milk on lipid profile, histopathology, and liver enzymes in animal model

Ali, A., Iqbal, S., *Sohaib, M., Khan, A. U., Younis, R. M. W. and Junaid, S. B.

Department of Food Science and Human Nutrition, University of Veterinary and Animal Science, Syed Abdul Qadir Jilani Outfall Road, Lahore-54000, Punjab, Pakistan

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Abstract

The pandemic of obesity and other diet-related non-communicable diseases are serious threats to the wellbeing and financial prosperity around the globe. It has been documented that the consumption of processed foods with high levels of refined sugars and fats are the main cause contributing to this phenomenon. The present work was therefore conducted to assess the effects of the consumption of non-dairy tea whiteners (NDTW's) on lipid profile, histopathology, and liver enzymes of rats for a period of three months. To this end, 30 albino Wister rats (7 weeks old, 130 ± 5 g) was divided into five groups namely control (G_1), fed with whole milk (G_2), and groups fed with defined dose of commercial NDTW's (G_3, G_4, and G_5). All rats were provided feed and water *ad libitum* during the experimental period. At trial completion, the rats were decapitated, and samples (blood, liver) were collected and subjected to blood chemistry (lipid profile) and liver histopathological analyses. Results indicated that as compared to milk, powder NDTW consumption increased the level of cholesterol (69%), triglycerides (98%), and LDL (84%), whereas HDL was non-significantly affected. On the other hand, liquid NDTW consumption led to the increase in triglyceride levels for all NDTW, and yielded 130% increase as compared to milk, which might have been attributed to composition of NDTW. Additionally, liver and renal functional tests yielded non-significant results. The present work concluded that the consumption of NDTW contributed towards derangement and elevation in lipid profile parameters as compared to milk, particularly if consumed for longer period of time.

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Introduction

Non-dairy tea whiteners (NDTW's) or tea-whiteners are commercialised products used as an alternative to milk due to their whitening ability and absence of lactose. NDTW's are available in both powder and liquid forms (Ajmal *et al.*, 2019). These NDTW's are used to make cold or hot beverages such as tea and coffee. The need for developing a dairy-like product with extended shelf life came during World War II in order to overcome the shortage of dairy products so that the armed forces could use it for longer periods. These products were economical as compared to dairy products. So, at that time, these non-dairy products made special places in the market, and were available on almost every coffee vending machine. Mocha Mix was the first NDTW in the world launched in California, USA back in 1950's. Afterwards, Mocha Mate, Coffee Rich, and Silk Soymilk creamers were developed in 1956, 1961, and 1999, respectively (Shurtleff and Aoyagi, 2013).

Next to water, tea and coffee are the most widely consumed beverages around the world. These beverages can be consumed with or without adding dairy, as well as NDTW's. The NDTW's are produced and available almost all over the world, and developed using vegetable fat (mostly palm oil), milk solids, sugars, sodium caseinate, emulsifiers, and stabilisers (Huang *et al.*, 2019). To replicate the mouthfeel of milk fats, NDTW's often contain partially or fully hydrogenated vegetable-based fats (Srikaeo and Singchai, 2016).

In terms of NDTW's composition, the fat contents of powder and liquid NDTW's are 25 - 50 and 3 - 18%, respectively. The protein contents of powder and liquid NDTW's are between the range of 3 - 12 and 1 - 3%, respectively. Powder NDTW contains 35 - 65% of carbohydrates, and liquid

*Corresponding author.
Email: muhammad.sohaib@uvas.edu.pk

NDTW contains 2.5 - 6% of carbohydrates (Leuenberger *et al.*, 2015; Ayna *et al.*, 2019). As compared to NDTW's, milk is a complete diet, containing almost all macronutrients, vitamins, minerals, and some antimicrobial / active ingredients. As compared to NDTW's, buffalo and cow's milk contain 6.89 and 3.75% of fats, respectively, which is considerably lower than the amount of fats available in NDTW's. The carbohydrate content of milk is only 5.18% (Fahmid *et al.*, 2016; USDA, 2018). Major fat component of NDTW is saturated fat which is approximately 98.71% of total fat. Bovine milk contains a variety of fat including 67% saturated fatty acid, 26% mono-unsaturated fatty acid, and 2% poly-unsaturated fatty acid (USDA, 2018). Processed products made using refined nutrients like palm oil (PO), either partially or fully hydrogenated PO, sugar, and other refined nutrients, may increase the risk for development of non-communicable diseases such as cardiovascular diseases (CVD), diabetes, and hypertension if consumed for prolonged period (Misra *et al.*, 2017; Aung *et al.*, 2018; Kadandale *et al.*, 2019).

The NDTW's are intended to be used for making hot and cold beverages like tea and coffee, but the general population in the sub-continent is consuming these products as a replacement and substitution of milk, mainly owing to the cheaper price, lack of nutritional knowledge, as well as week regulatory framework regarding these non-dairy products. Not only that they are consuming it as milk, but also feeding it to the infants as milk source due to the lack of nutrition awareness / knowledge; and excessive advertisement on the electronic media is one of the major concerns as these NDTW's do not provide the required nutrients, as well as the presence of trans fatty acids in these NDTW's also contribute to detrimental effect on the child / infant health. This can also lead to malnutrition among infants as NDTW's have low nutritional value as compared to breast milk and buffalo / cow's milk (Huang *et al.*, 2019). To the best of our knowledge, there are no scientific studies available on the effects of these NDTW's on different health parameters, especially lipid profile, which is one of the major indicators for non-communicable diseases. Therefore, the present work was conducted to assess the effects of consumption of NDTW's on lipid profile, histopathology, and liver enzymes in comparison to milk, using an animal model.

Materials and methods

Chemicals and reagents

All chemicals used were of analytical grade, and purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated. Analytical kits for Cholesterol FS 10 (Cat no. 113009910923), Glucose GOD FS 10 (Cat no. 125009910923), Alanine Transaminase (ALAT) (GPT) FS (IFCC mod.) (Cat no. 127019910920), Creatinine FS (Cat no. 117119910920), High Density Lipoprotein-C Immuno FS (Cat no. 135219910920), Triglycerides FS (Cat no. 157109910923), Alkaline Phosphatase (Cat no. 104419910920), and Low-Density Lipoprotein-C Selectra (Cat no. 141219910921) were purchased from Response®-Diagnostic Systems, Holzheim-Germany. Feed material and NDTW's were purchased from local markets.

Proximate analysis of NDTW's and milk

Proximate analysis of NDTW's was performed following the Association of Official Analytical Chemists method (AOAC, 2006) including moisture (%) and dry matter (method 934.01), crude protein (method 984.13), crude fat (method 920.39), crude fibre (method 978.10), ash (%) (method 942.05), as well as nitrogen free extract (NFE) that is actually the indicator of the carbohydrate.

Animal procurement, housing, and handling

Male Wister rats (n = 30) with weight of 130 ± 5 g and aged six weeks were purchased from University of Veterinary and Animal Sciences, Lahore. These rats were fed with basal diet for one week before starting the animal trial for acclimatisation. All rats were housed in animal room with controlled temperature (25 ± 2°C) under 12-hour cycle of light and dark. All the methods and procedures for rat trial, handling, sampling, and analyses were approved by the animal ethical committee of the Office of Research, Innovation and Commercialisation, University of Veterinary and Animal Sciences, Lahore, Pakistan (reference no.: DR/ 550; date: 14-05-2018).

Experimental design

For animal trial, the rats (n = 30) were divided into five different groups (each having equal rats per group), namely G_1 = control group, fed with water along with basal diet only; G_2 = group fed with whole milk along with basal diet, NDTW; G_3 = group fed with powder NDTW along with basal diet; G_4 and G_5 = groups fed with different brands of liquid NDTWs along with basal diet. All groups received Miller Bander Basal feed *ad libitum* during this trial (Miller and Bender, 1955). The doses of liquid and powder NDTW's were calculated using human equivalent dose equation (HED (mg/kg) = animal dose (mg/kg) × [(animal weight (kg)/ human weight (kg)) × 0.33].

The HED for liquid NDTW was 30 mL/cup (for 70 kg human), thus for three cups it would be 90 mL. By translating this HED into animal dose (mg/kg), the dose of 1.33 mL/130 g of rat was calculated for liquid NDTW. Similarly, HED for powder NDTW was 8 g/cup (70 kg human), thus for three cups it would be 24 g. By translating this HED into the animal dose (mg/kg), the dose of 342 mg/130 g of rat was calculated for powder NDTW's. Animal dose of 342 mg was dissolved in 1.33 mL of distilled water (same volume calculated for liquid NDTW), and given to rats from powder NDTW's group. The dose of milk was calculated using same calculations as liquid NDTW in aforementioned procedure (Reagan-Shaw *et al.*, 2008). The doses of milk and NDTW's were given orally using tube feeding method for 12 weeks, whereas equal amount of water was given to control group to equalise stress. The doses were calculated considering the body weight of rats on weekly basis, as mean weight (g) of all groups. After 12 weeks, all rats were euthanised by following the standard protocols described in Boubaker *et al.* (2018), and blood sampling was done using heart puncture technique (Hoggatt *et al.*, 2016).

Monitoring parameters

The body weight change and feed intake of rats were monitored on weekly basis using an electronic balance (UX 420H, Shimadzu, Japan), whereas water intake was also monitored on daily basis to access the impact of NDTW's consumption on the said parameters in rats.

Blood chemistry analysis

After blood sampling serum was separated, all serum analysis was performed using blood chemistry analyser (Response® 910, Diagnostic System-SIEMENS, Germany) following these parameters: low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride (TG), total cholesterol, alkaline phosphatase (ALP), alanine amino transferase (ALT), creatinine, albumin, and total protein. Serum chemistry data was analysed using software version 2.2.3.3.

Histopathological analysis

After the dissection of rats, the liver was separated and stored in 10% neutral buffered formalin. Histopathological samples were processed in the following manner, including dehydration, clearing, and infiltration. Tissues of 0.4 µm thickness were cut after preparation of blocks. H&E staining of tissue sections was performed following the methods described in Bancroft and Gamble (2008).

Statistical analysis

Data were analysed using SPSS version 25. One-way analysis of variance (ANOVA) was performed to assess the significant parameters. Duncan Multiple Range test (DMRt) was performed on the significantly different parameters to assess differences between the groups, and were considered significance at $p < 0.05$ by following the guidelines of Steel and Torrie (1997).

Results

The proximate compositions of the different NDTW's and milk samples assessed in the present work are shown in Table 1. It was observed that the protein content of milk, also NDTW2 and NDTW3 were 3.52 ± 0.28, 1.27 ± 0.15, and 1.23 ± 0.25, respectively, whereas NDTW1 was 15.83 ± 0.59. Similarly, the carbohydrate content was observed significantly higher in whole milk and NDTW3 as compared to NDTW2 (53.97 ± 0.85 in NDTW 1, 4.27 ± 0.25 in NDTW 2, 5.37 ± 0.42 in NDTW 3, and 5.55 ± 0.31 in milk samples). The protein content was significantly lower in NDTW2 and NDTW3 as compared to whole milk (15.83 ± 0.59, 1.27 ± 0.15, 1.23 ± 0.25, and 3.52 ± 0.28 in NDTW 1, NDTW 2, NDTW 3, and whole milk, respectively). Similarly, significantly high amount of fat was found in NDTW2 and NDTW3 as compared to whole milk, with the highest in NDTW1 (27.33 ± 0.65) and lowest in milk (3.70 ± 0.20). Ash contents were significantly high in whole milk as compared to the other groups (0.37 ± 0.02, 0.40 ± 0.02, 0.45 ± 0.04, and 0.63 ± 0.04 in NDTW 1, NDTW 2, NDTW 3, and whole milk, respectively) (Table 1).

At the baseline, all groups had significantly same weight, but at the end of the trial, significantly higher (p = 0.001) weight gain was reported, with control, whole milk, and NDTW1 resulting with 259.67 ± 22.54, 268.00 ± 29.15, and 259.33 ± 27.62, respectively, as compared to NDTW2 (205.33 ± 17.74) and NDTW3 (197.33 ± 22.26). The total weight gain observed in control, whole milk, and NDTW1 were 130.17, 136.0, and 129.08 g, respectively. The lowest weight gain of 77.33 and 69.58 g were observed in NDTW2 and NDTW3, respectively (Table 2).

The effect of different NDTW's consumption on liver enzymes was found non-significant for serum creatinine, albumin, and total protein as compared to control and whole milk (Table 3).

Table 1. Proximate compositions of multiple commercial NDTW's and whole milk.

Composition (g/100 g or mL)	NDTW1	NDTW2	NDTW3	Whole milk	*p*-value
Carbohydrate	53.97 ± 0.85	4.27 ± 0.25[b]	5.37 ± 0.42[a]	5.55 ± 0.31[a]	0.007
Protein	15.83 ± 0.59	1.27 ± 0.15[b]	1.23 ± 0.25[b]	3.52 ± 0.28[a]	0.001
Fat	27.33 ± 0.65	6.50 ± 0.17[a]	6.20 ± 0.36[a]	3.70 ± 0.20[b]	0.001
Ash	0.37 ± 0.02	0.40 ± 0.02[b]	0.45 ± 0.04[b]	0.63 ± 0.04[a]	0.001
Dry matter	97.51 ± 0.39	12.43 ± 0.31	13.25 ± 0.55	13.40 ± 0.47	0.083

Values are mean ± SD. Means followed by similar lowercase superscripts in the same row do not differ significantly ($p > 0.05$). NDTW1 (powder) was excluded before applying statistical analysis in order to compare liquid NDTW groups with whole milk. NDTW: non-dairy tea whitener.

Table 2. Weight gain pattern of rats fed with multiple commercial NDTW's and whole milk after 12 weeks.

Group	Baseline (g)	Week 12 (g)	Weight gain after 12 weeks (g)	% weight gain from base line
Control	129.50 ± 4.65	259.67 ± 22.54[a]	130.17	101
Whole Milk	132.00 ± 4.76	268.00 ± 29.15[a]	136.00	103
NDTW1	130.25 ± 4.99	259.33 ± 27.62[a]	129.08	99
NDTW2	128.00 ± 3.83	205.33 ± 17.74[b]	77.33	60
NDTW3	127.75 ± 3.40	197.33 ± 22.26[b]	69.58	54

Values are mean ± SD. Means followed by similar lowercase superscripts in the same row do not differ significantly ($p > 0.001$). NDTW: non-dairy tea whitener.

Table 3. Effect of consumption of commercial NDTW's and whole milk on liver enzymes, creatinine, albumin, and protein level of rats subjected to the study.

Enzyme level	Control	Whole milk	NDTW1	NDTW2	NDTW3	*p*-value
Alanine aminotransferase (U/L)	42.42 ± 2.13	32.68 ± 9.28	38.08 ± 3.41	38.11 ± 16.41	42.83 ± 13.25	0.578
Alkaline phosphatase (U/L)	93.28 ± 22.39	87.20 ± 24.71	134.53 ± 32.00	80.96 ± 24.17	97.69 ± 28.73	0.062
Creatinine (mg/dL)	0.62 ± 0.13	0.58 ± 0.08	0.67 ± 0.06	0.55 ± 0.06	0.68 ± 0.07	0.130
Protein (mg/dL)	12.08 ± 4.84	10.00 ± 6.26	4.00 ± 1.11	11.76 ± 6.04	7.98 ± 3.73	0.135
Albumin (mg/dL)	12.10 ± 2.82	12.40 ± 6.67	8.75 ± 2.89	8.58 ± 2.94	7.22 ± 2.51	0.191

Values are mean ± SD. NDTW: non-dairy tea whitener, U/L: unit per litre, mg/dL: milligram per decilitre.

Table 4 shows the effect of consumption of NDTW's on various lipid profile parameters. Significant increase in cholesterol was observed in NDTW 1 (54.00 ± 14.14) as compared to control (39.20 ± 8.11), whole milk (32.00 ± 2.74), and NDTW2 (32.80 ± 7.50). NDTW3 had significantly similar total cholesterol levels (42.50 ± 10.62) as compared to all other groups. Triglycerides levels were significantly higher among NDTW1 (49.50 ± 14.66) and NDTW3 (57.40 ± 7.57) as compared to control (28.00 ± 5.70), whole milk (25.00 ± 7.07), and NDTW2 (30.00 ± 16.49). LDL levels were significantly higher in NDTW1 (22.01 ± 7.72) as compared to whole milk (11.97 ± 3.09), NDTW2 (10.12 ± 3.59), and NDTW3 (12.79 ± 3.90). HDL remained non-significant throughout the trial, and the values were 16.88 ± 2.91, 14.35 ± 4.28, 20.98 ± 5.16, 17.80 ± 3.61, and 23.74 ± 7.07 for control, whole milk, NDTW1, NDTW2, and NDTW3, respectively (Table 4).

Figures 1A and 1B show normal hepatocytes of rats from control group fed with basal diet, plain water, and milk, respectively. Meanwhile, Figures 1C, 1D, and 1E show enlarged hepatocytes with deformed boundaries and vacuole formation in NDTW1, NDTW3, and NDTW2 groups, respectively.

Table 4. Lipid profile including cholesterol, low density lipoprotein, high density lipoprotein, and triglycerides of rats fed with commercial NDTW's and whole milk.

Group (mg/dL)	Control	Whole milk	NDTW1	NDTW2	NDTW3	*p* value
Cholesterol	39.20 ± 8.11^{b}	32.00 ± 2.74^{b}	54.00 ± 14.14^{a}	32.80 ± 7.50^{b}	42.50 ± 10.62^{ab}	0.013
Triglycerides	28.00 ± 5.70^{b}	25.00 ± 7.07^{b}	49.50 ± 14.66^{a}	30.00 ± 16.49^{b}	57.40 ± 7.57^{a}	0.001
LDL	15.79 ± 4.30^{ab}	11.97 ± 3.09^{b}	22.01 ± 7.72^{a}	10.12 ± 3.59^{b}	12.79 ± 3.90^{b}	0.011
HDL	16.88 ± 2.91	14.35 ± 4.28	20.98 ± 5.16	17.80 ± 3.61	23.74 ± 7.07	0.053

Values are mean ± SD. Means followed by similar lowercase superscripts in the same row do not differ significantly ($p > 0.05$). NDTW: non-dairy tea whitener, mg/dL: milligram per decilitre.

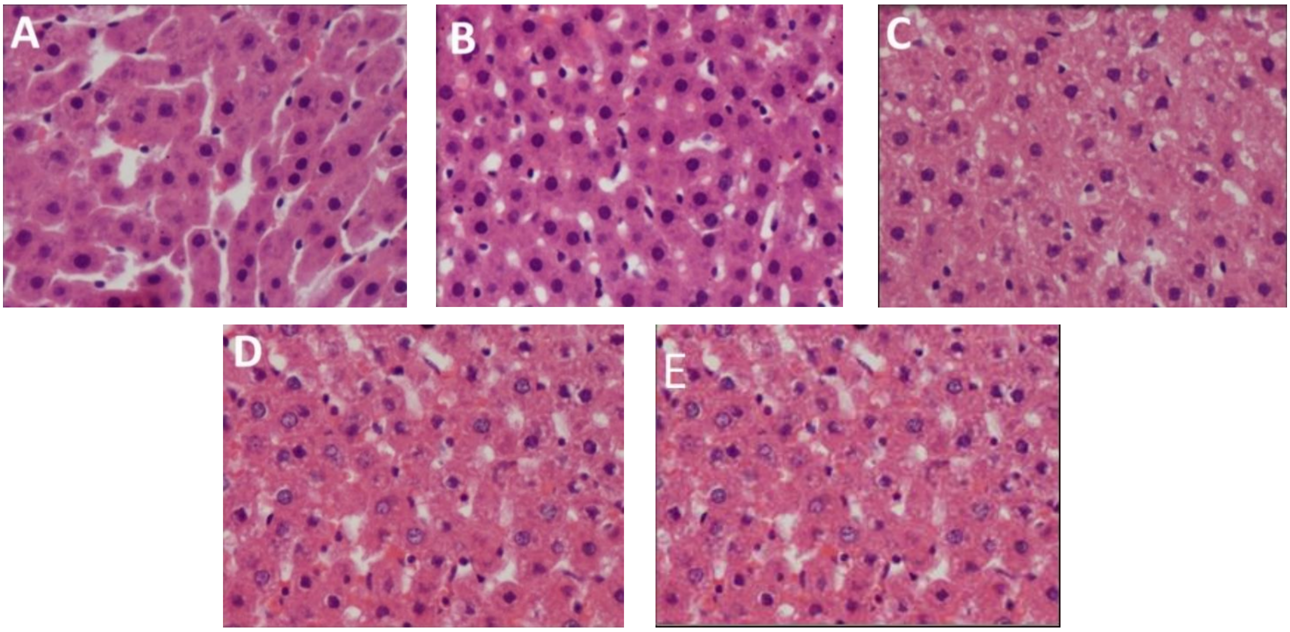

Figure 1. Hepatic histopathology of rats fed with NDTW's, milk, and plain water at 100× magnification, and dyed with haematoxylin and eosin stain. **(A)** and **(B)** show normal hepatocytes of rats fed with water (control group) and whole milk (milk group), respectively. **(C)**, **(D)**, and **(E)** show fatty vacuole accumulation in hepatocytes (enlarged with deformed boundaries) of NDTW1, NDTW3, and NDTW2, respectively.

Discussion

The disturbance in lipid profile is triggered by high amount of saturated fat and refined carbohydrate ingestion through diet that can lead to the development of CVDs and other related complications. In the present work, different rat groups fed with NDTW's and their impact was evaluated in some parameters including lipid profile, histopathology, and liver enzymes for three months research trial as compared to control and whole milk fed group. Results demonstrated that the consumption of NDTW's elevated lipid profile parameters including total cholesterol, LDL cholesterol, and triglycerides. As there is no published literature describing direct NDTW consumption effects on the lipid profile in animal or human model, it is highly difficult to correlate these effects with the lipid profile. Also, the effects of NDTW on elevated lipid profile in rats may be attributed to the levels, types, and compositions of fats, as well as higher sugar level and refined carbohydrates present in these food products intended for consumption as tea-based products.

A group of researchers assessed the fatty acid composition of various UHT treated products (tea whiteners / milk / dairy drinks), and observed that trans fatty acid (C18:1) concentration of 18.1 - 18.5% among liquid NDTW, which is one of the major contributors of higher blood cholesterol level in humans (Ajmal *et al.*, 2019). On the other hand, trans fatty acid contents (%) of goat, sheep, and cow milk are 0.45, 0.76, and 0.57, respectively, that are comparatively lower as compared to liquid NDTW (Markiewicz-Kęszycka *et al.*, 2013). Additionally, milk contains a variety of fatty acids ranging from carbon chain with length of 4 - 18, which are helpful for optimal growth, as well as the presence of low levels of trans fatty acids, thus making it suitable for optimal human health. The NDTW's contain high trans fatty acids and low short-chain fatty acids; therefore, the consumption of these food products can result in hyperlipidaemia (Ajmal *et al.*, 2019).

Similarly, another study was conducted involving 1,088 children (8 - 11 years) to assess the effects of intake of whole milk and reduced fat / skimmed milk on lipid profile. Results reported that children with normal levels of triglycerides and HDL consumed more whole milk as compared to the ones consuming reduced fat or skimmed milk. The study also reported that whole milk consumption was also associated with reduced body mass index (BMI), fat mass, and fat mass index (Lahoz-García *et al.*, 2019), which correlates with our findings that observed whole milk consumption helped to improve lipid profile of rats in managing hypercholesteremia. The possible reason behind inverse relation of dairy products and metabolic syndrome is because of the ingredients (calcium, proteins, and fats). Calcium can bind free fatty acids and bile salts, thus reducing the absorption of cholesterol in the intestine. Moreover, proteins can also increase the satiety level for long or shorter periods, and reduce total energy intake, which is helpful for achieving optimal health in living organisms (Bachmanov *et al.*, 2016).

The frequent consumption of food products rich in trans fatty acids and refined carbohydrates are linked to the development of obesity and type II diabetes mellitus. Studies also suggest sweet beverage consumption can also trigger childhood obesity, which on the longer run, contribute towards weight gain, CVD's, and other non-communicable diseases (Demmler *et al.*, 2017; Rauber *et al.*, 2018). According to international report in 2018, the NDTW market will rise up to 3.7 million tons by 2025, which will have a consequence in terms of nutritional problems if products are not used as per their intended use. Moreover, powder NDTW's contained high fats (27.33%) and carbohydrates (53.97%), while liquid NDTW's contained low fats (6.5%) and carbohydrates (≈4 - 5.5%) as indicated by proximate analysis. On the other-hand, whole milk contained only 5.55% carbohydrates and 3.7% fats, which was significantly less than powder and liquid NDTW's (Table 1). The composition of NDTW's is similar as described in Ayna *et al*. (2019) and Leuenberger *et al.* (2015). Also, another study was conducted on composition analysis of NDTW's, milk, and dairy drink to assess the fatty acid composition and antioxidant potential in relation to storage period reported (%). Results elaborated that liquid NDTW's contained 6.00 ± 0.03 fat, 3.28 ± 0.07 proteins, and 4.68 ± 0.13 lactose, whereas the levels of fat, protein, and lactose reported for the whole milk was 3.50 ± 0.03, 3.28 ± 0.07, and 4.68 ± 0.13, respectively. On the other hand, nutritional composition of dairy drink reported 3.50 ± 0.03 of fat, 3.28 ± 0.07 of protein, and 4.68 ± 0.13 of lactose (Ajmal *et al.*, 2019). Major fat component of the NDTW is saturated fat which is approximately 98.71% of total fats. These products when consumed in high amount for a prolonged period can cause disturbance in the lipid profile parameters (Katulanda *et al.*, 2018).

Furthermore, sedentary lifestyle, high carbohydrate and saturated fat diets, along with diets low in omega-3 polyunsaturated fatty acids are major determinants of dyslipidaemia in Asian Indians (Leuenberger *et al.*, 2015; Ayna *et al.*, 2019). Dyslipidaemia can be a risk factor for development of multiple non-communicable diseases such as CVD's, hypertension, atherosclerosis, and diabetes mellitus due to the disruption of endothelial dysfunction and inflammation (Gebreyes *et al.*, 2018).

As compared to NDTW's, a meta-analysis performed on dairy products and CVD's concluded that dairy products have protective effect on CVD's (Bachmanov *et al.*, 2016), which was also demonstrated by the present work which reported that even after 12 weeks, whole milk feeding group had significantly lower lipid profile as compared to NDTW's group (Table 4).

The NDTWs are developed using vegetable fat (mostly PO), milk solids, sugars, sodium caseinate, emulsifiers, and stabilisers (Rosida *et al.*, 2016), and trans fatty acid of these products is also one of the big concerns if the industry uses low quality fats containing higher level of trans fatty acid. High intake of saturated fatty acid is directly linked to the elevated cholesterol which is linked to the increase in coronary heart diseases (CHD). Primary cholesterol elevating fatty acids are present in PO *e.g.* lauric acid (12 carbon chain), myristic acid (14 carbon chain), and palmitic acid (16 carbon chain) (Afonso *et al.*, 2016). Moreover, World Health Organization conclusively reported that palmitic acid consumption can lead to increased CVD's (Fatima *et al.*, 2019). Also, palmitic acid elevates the blood cholesterol level as well as high carbohydrate diet (Silva-Santi *et al.*, 2016). Even after consuming products made by hydrogenated vegetable fats (specifically PO) *e.g.*, NDTW's (three cups per day) for 12 weeks could elevate lipid profile as compared to whole milk / dairy source (Table 4). As compared to myristic and lauric acid, palmitic acid increases ratio of total cholesterol:HDL (Chen *et al.*, 2011; Odia *et al.*, 2015; Ismail *et al.*, 2018).

In the present work, enlarged hepatocytes with deformed boundaries and vacuole formation were observed among NDTW's groups as compared to control and whole milk group after 12 weeks (Figure 1), and these findings are supported by a study conducted to assess the potential effects of various dairy products, including whole milk, on liver histopathology. The histopathology findings were rated for 0 - 4, with respect to severity of histopathological changes and whole milk group rated 0 in histopathological findings, as no histopathological change was observed in whole milk group after four weeks (Abdel-Moemin *et al.*, 2008). Also, it is very important to note that NDTW consumption as replacement of milk is highly dangerous, and can result in severe histopathological changes in individuals. Therefore, there is a need to launch a campaign using mass media to raise consumers' awareness on the use of milk, and tea whitener industry should focus on the level of trans fatty acid control in these food products to protect consumers' health.

Trans fatty acids are unsaturated fat attributed to a number of negative health effects in living organisms, mainly created during hydrogenation process that converts liquid vegetable oils into semi-solid partially hydrogenated oil. Globally, actions are being taken regarding labelling and nutrition facts of trans fatty acid to reduce its intake and protect consumers health, and legislations regarding trans fatty acid are different among countries. In this regard, Denmark took proactive measures and banned products containing > 2% trans fatty acid leading to its reduced consumption among Danish population and resulting with decline (60%) in CVD's (Restrepo and Rieger, 2016; Wilczek *et al.*, 2017). Many other countries have also taken safety measures against trans fatty acid and forcing food industries towards its elimination, and in European countries, trans fatty acid intake is < 1%, which is no longer a health problem (trans fatty acid replacement solution). WHO also recommend governments to phase out partial hydrogenated fats to eliminate trans fatty acid in reducing the problem (Astrup *et al.*, 2019), and in 2013, they suggested that partially hydrogenated fats, which are considerable source of industrial trans fatty acid, as not generally recommended as safe (GRAS) for any use in food (Grossman, 2015). Unfortunately, in developing countries, legislations and recommendations were not implemented in true sense resulting with partially or fully hydrogenated fats in many of products *e.g.*, NDTW's, edible oils, butters, margarines, and bakery products. Additionally, low-income population groups, who owing to other lifestyle factors, already have an increased risk of

CHD and commonly eat products with high trans fatty acid contents (Sacks *et al.*, 2017) which can have detrimental effects on their health.

Conclusion

The present work demonstrated that as compared to milk, powder NDTW consumption led to 69% increase in cholesterol, 98% increase in triglyceride, and 84% increase in LDL levels. On the other hand, liquid NDTW consumption led to 130% increase in triglyceride levels as compared to milk. In a nutshell, the consumption of NDTW's may contribute towards the elevation of LDL, total cholesterols, and triglycerides as compared to dairy whiteners / milk. Additionally, further studies are required to assess the effects of NDTW consumption on other health parameters, and the findings are useful for consumers, health professionals, food industries, and regulatory agencies. Moreover, low caloric, low fat, and trans fatty acid-free dairy or tea whiteners can be a healthy choice and replacement for currently available NDTW's. In conjunction with this issue, media campaign is also needed to disseminate information and raise awareness on tea whitener commercial use as food product.

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