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Effect of High Dietary Consumption of Locally Available Ghee on Renal Function in Mice

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ABSTRACT

The study was designed to evaluate the detrimental effects of dietary consumption of Banaspati ghee on renal function of mice. Two locally manufactured brand of Banaspati ghee daily to mice at a rate of 10% of their daily food intake for a period of 4 weeks. After 4 weeks, both male and female mice were sacrificed and kidneys were taken out. Biochemical parameters e.g., lipid peroxidation (LPO), catalase (CAT), glutathione-S-transferase (GST), reduced glutathione (GSH) were determined to evaluate oxidative stress while urea and creatinine, was used to evaluate kidney function. It was found that there was an increase in weight ($p<0.05$) of both males and female mice fed with diet containing ghee. Kidney LPO and CAT were significantly increased in both male and female mice. GSH level showed decreasing trend in female while increase was observed for male kidney. Creatinine level increased in both male and female experimental groups compared with control. In the light of present results, it was concluded that high daily intake of ghee available in market can cause damage to vital organs like kidney. In general female physiological system seems to be more prone to damage caused by these diets as compared to male physiological system.

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Authors' Contribution
DAB and KPL designed the study. SA performed experiments. MF analyzed the results. SA and DAB wrote the article.

Key words
High-fat-diet, Renal effects, Oxidative stress, Lipid peroxidation, Antioxidant enzymes

INTRODUCTION

Uncontrolled consumption of high fat diet interferes with many metabolic processes and cause cellular and molecular damage (de Paula *et al*., 2016). High consumption of refined foods containing higher carbohydrate and fats contributes to the epidemic of obesity and also linked with oxidative damage in many organs of the body and also caused impaired auditory functions (Kartha *et al*., 2008; Bruce *et al*., 2009; Yokota *et al*., 2009; Ballal *et al*., 2010; Gopinath *et al*., 2011; Spankovich *et al*., 2011). It results in many health complication (diabetes type 2 and hypertension) and cause damage to vital organs like heart, liver (Manes *et al*., 1973; Van Gaal *et al*., 2006) kidneys, lungs, colon (DeClercq *et al*., 2015), skin (Gallagher, 2005) and brain (Cazettes *et al*., 2011). Obesity results when intake of dietary fats (calories) are higher than the daily requirements of the body. These excessive calories are stored and end into obesity and other related diseases. Moreover, life style of lesser physical activity and eating food rich in fat are major cause of fat storage in the body.

Recently large number of studies reported a strong association between high fat diet and chronic kidney disease (CKD) (Palaniappan *et al*., 2003; Chen *et al*., 2004; Kurella *et al*., 2005; Ninomiya *et al*., 2006). Obesity resulted in fat deposits near kidney which cause stress and effecr renal sodium reabsorption. Obesity can directly affect the renal vasodilatation and hyper filtration of glomerulus; it is a special kind of process that conserve sodium balance regardless of the tubular reabsorption and affect the arterial blood pressure. In addition, obesity also caused other issues such as inflammation and oxidative stress which might end up into renal failure.

In Pakistan, ghee (heated clarified milk fat) has been used as a preferred source of fat in all domestic and commercial cooking. The traditional ghee (locally known as desi ghee) is made from butter oil cannot fulfill the ever increasing demand, therefore, alternatives were sought to fulfill the vacuum of supply and demand. Henceforth the substitute of ghee was provided in the form of hydrogenated vegetable oil (locally known as Banaspati ghee) emulating the texture and flavor of the original butter oil ghee. In converting the vegetable oil into ghee, tin is also used as a catalyst for hydrogenation. This process induces trans-fatty acids in the final product. In order to make the end product more appealing to the poor masses, industry looked for cheaper alternatives of vegetable oil. Now a day, a blend of palm oil, coconut oil, rapeseed oil, cotton seed and soya bean oil is frequently used without any concern regarding quality assurance and public health. A lot of brands are now available in market that offers ghee at lower rates for human consumption under the general name of Banaspati

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ghee. In Pakistan, people consume great amount of ghee every day in the form of fried food. These vegetables ghees which are cheaply available in Pakistan cause different type of liver, kidney, and heart diseases.

In December, 2018, a survey by the provincial food authority (PFA) declared 61 different types of edible ghee and cooking oils to be unsafe for human consumption. PFA teams also confiscated 29,099 liters of unhygienic ghee and oils from different zone of Lahore. In view of above mentioned points, preliminary study was designed to investigate the effects of two brand of commonly available ghee on renal function using a mice model.

MATERIALS AND METHODS

Two types of ghee samples (Dil Dil and Adil) were collected from Sandhaa and Anarkali areas of Lahore, Pakistan. Ghee samples were collected in small clean plastic boxes and refrigerated until used for experiment. Diet was prepared by mixing 10g of ghee to the normal mice chow. Two experimental diets were prepared for two samples. Diet-1 contained 10% of Dil Dil and diet-II contained 10% of Adil. Control groups were fed with mice chowder alone.

Three week-old 15 male and 15 female albino mice were purchased from the veterinary research institute (VRI) and brought to Government College University, Lahore. All the mice were housed in the animal housing facility of Government College University, Lahore under controlled environment. In the lab all the rodents had free availability to drinking water and regular chow diet. After a week of acclimatization, the mice were randomly divided into six different groups (n= 5 each group) namely; control female; control-male. Experimental group-I fed with diet 1, experimental group II fed with diet 2. Feeding trial was conducted for 4 weeks. Bedding were replaced every 3 days and mice were weight weekly.

Proximate composition of diet

Mice chowder and both experimental diets were analyzed for total moisture, ash, fat fiber, protein and total energy using standard protocols in duplicate.

Sampling of tissues

After 4 weeks, mice were anesthetized, weighed and dissected. Kidneys were removed, cleared of any fat and weighed. One kidney was stored in formalin for routine histologic studies and other kidney was washed with 0.9% ice-cold saline solution blotted on filter paper and stored at -80°C, till use for analysis of biochemical tests. Experimental protocols and animal handling were permitted by research committee of Zoology Department, Government College University Lahore, Pakistan.

Biochemical analysis

Kidney tissue was grounded in liquid nitrogen and later homogenized in phosphate buffer (0.1 M, pH=7.4) using a glass homogenizer and centrifuged at 13000 rpm (30 min at 4 °C) for post-mitochondrial supernatant (PMS). Oxidative stress parameters *e.g.,* catalase activity, Glutathione-S-transferase activity, reduced glutathione content and lipid peroxidation was measured according to protocol described earlier by Faheem and Lone (2017d). Protein content was estimated by using Bradford reagent as described by He (2011) with bovine serum albumin as standard. Kidney function parameters such as urea and creatinine was measured using commercially available kits (Randox).

Histological analysis

The formaline-fixed tissues were dehydrated in various grades of ethanol, cleared in xylene and impregnated with wax (mp; 58°C). Five microns thick sections were cut using rotary microtome (Leica RM 2165). Tissue sections were stained with haematoxyline and eosine and PAS stains. Stained slides were studied and photographed by high resolution microscope (Leica, Japan) fitted with a digital camera.

Data analysis

Data were analyzed using Graphpad prism 7. One way analysis of varience (ANOVA) followed by Tukey's Post Hoc test was used to determine difference between means. Data were expressed as mean ± S.E.M.

RESULTS

Proximate analysis of feed

The proximate composition of normal and experimental diet is given in Table I. There is an increase in fat content in experimental diets. Total energy also increased in both the experimental diets.

Body weight gain

A significant increase in body weight was recorded in male fed with diet-II after 4 weeks. However, weight gain in females was significantly increased in both experimental groups (Fig. 1).

Histopathological analysis of the kidneys

The renal cortex of the control rats contained glomeruli, vessels, tubules and interstitium. When evaluating these renal specimens by light microscopy on an H-E-stained section, the following glomerular features

Table I. Proximate composition of control and experimental diets.

Groups	Moisture (%)	Ash (%)	Fiber (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Total energy (kcal/g)
Control mice chow	6.74±0.273	3.89±0.150	1.54±0.273	15.75±0.242	2±0.311	70.06±0.343	430.07±0.345
Experimental diet-I	5.28±0.081**	0.80±0.0288****	2.49±0.0811***	19.90±0.176****	4.33±0.105**	67.17±0.308**	465.20±0.702****
Experimental diet-II	3.73±0.105****	1.22±0.0702****	6.34±0.105****	19.14±0.0416****	4.67±0.212**	64.86±0.277****	455.96±0.871****

Data expressed as mean± S.E.M. *=P<0.05; **=P<0.01; ***=P<0.001; ****=P<0.0001.

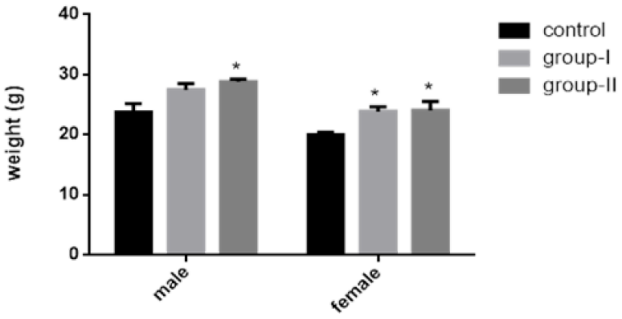

Fig. 1. Average weight gain in control and experimental groups fed with normal mice chow and experimental diets respectively. Data expressed as mean ± S.E.M. *=P<0.05.

were seen: the overall cellularity of the glomerulus enclosed by a visceral layer and a parietal layer enclosing a urinary space in between, proximal convoluted tubules lined with brush border, distal tubules with characteristic cuboidal cells (Fig. 2a). H and E staining of kidney sections of mice fed with locally made Banaspati ghee showed various anomalies including dilation of blood vessels and irregular glomerulus with degenerated parietal layer. Degeneration and necrosis of interstitial tissue, tubules and glomerulus was also observed in the kidney sections of mice fed with experimental diets 1 and 2. Additionally, vacuole formation was also observed in experimental groups (Figs. 2b, c).

Biochemical analysis

The measured levels of lipid peroxidation, expressed as concentration of thiobarbituric acid reactive species (TBARS), in kidney tissue of male and female mice are shown in Figure 3. Dietry inclusion of locally made ghee resulted in significant ($p<0.05$) increase in LPO. In males, mice fed with diet-II induced significant increase in LPO (16.04 in control vs 53.40 nmol of TBARS formed/g tissue in group-II) while in females, both diets increased significant LPO (131.3 and 145.5 nmol of TBARS formed/g tissue) compared to control (40.6 nmol of TBARS formed/g tissue).

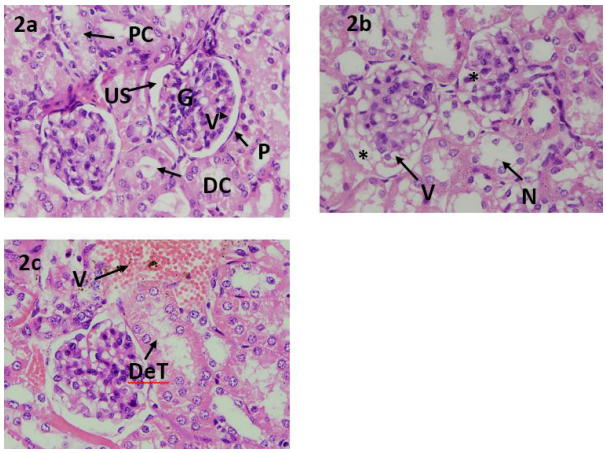

Fig. 2. (A) Photomicrograph of mice kidney from control group. PCT: proximal convoluted tubules; G: glomerulus; VL: visceral layer; PL: parietal layer; US; urinary space; DCT: distal convoluted tubules, (B & C) Photomicrograph of mice kidney fed with experimental diets. V: vacuolization; N: necrosis; DeT: degenerated tubules; VC: vascular congestion.

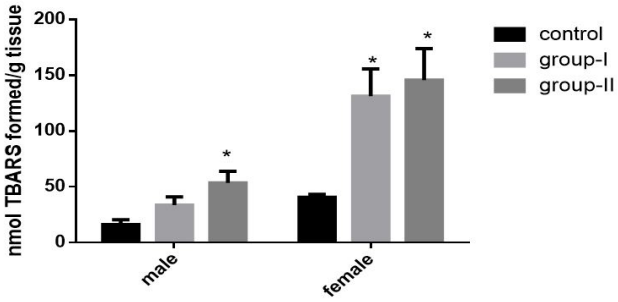

Fig. 3. Lipid peroxidation in mice kidney fed with 10% of ghee for 4 weeks. The values given are mean ± S.E.M.

Reduced glutathione content

Figure 4a shows the reduced GSH content in the kidney following 4-week dietary intake of ghee decrease non-significantly in male of group-II (1.9 nmol GSH/g of tissue in control vs 1.85 nmol GSH/g of tissue in group-II). In females, there was non-significant increase in GSH

content in both the experimental groups (2.08 nmol GSH/g of tissue in control vs 2.26 and 2.32 nmol GSH/g of tissue in group-I and II, respectively).

Catalase activity

A significant decrease ($p<0.05$) was observed in kidney catalase activity in male group fed with diet-I for 4-weeks. A highly significant decrease in catalase activity ($p<0.001$ and $p<0.0001$) was recorded for female mice fed with experimental diets-I and II (145.24 µmole H2O2/ mint/mg of protein in control vs 51.67 and 33.37 µmole H_2O_2/mint/mg of protein) (Fig. 4b).

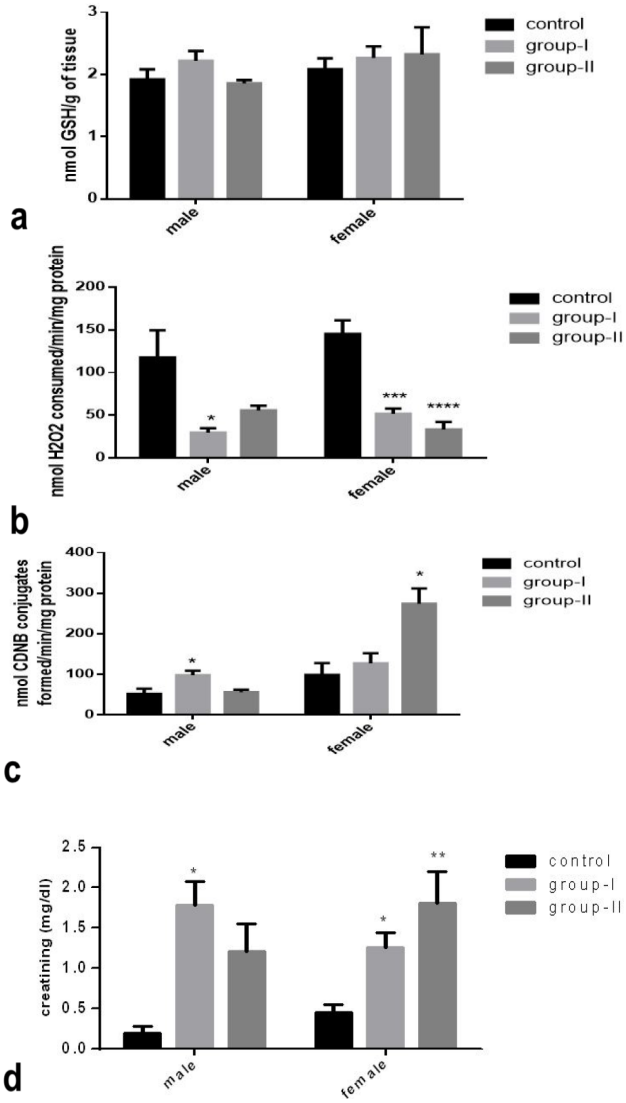

Fig. 4. (a) Glutathione level, (b) Catalase activity, (c) Glutathione-S-transferase activity and (d) Creatinine level in mice kidney fed with 10% of ghee for 4 weeks. The values given are mean ± S.E.M.

Glutathione-S-transferase activity

Glutathione-S-transferase activity was significantly increaed in male mice fed with diet-I (52.41 CDNB conjugate/ min/mg protein vs 98.12 nmol CDNB conjugate/ min/mg protein) and female mice fed with diet-II compared to the respective negative control mice (99.19 CDNB conjugate/ min/mg protein in control vs 274.62 CDNB conjugate/ min/mg protein in group-II) (Fig. 4c).

Creatinine levels

Creatinine level increased significantly in male fed with diets I however, in females, both diets induced statistically significant creatinine level (Fig. 4c).

DISCUSSION

In the present study, possible effects of daily consumption of local ghee were evaluated on kidney health using a mice model. Parameters such as kidney histology, oxidative stress indices and renal function tests were measured. High fat diet (HFD) is positively correlated with, obesity, insulin resistance (Matsuzawa-Nagata *et al*., 2008), diabetes, coronary heart disease (DiNicolantonio *et al*., 2016) and renal dysfunction (Deji *et al*., 2009; Garcia *et al*., 2018). Oxidative stress caused by dietary HFD was considered as the major cause of these metabolic dysfunctions (Matsuzawa-Nagata *et al*., 2008; de Paula *et al*., 2016; Echeverría *et al*., 2018).

Altered kidney histology and renal dysfunction has been associated with high fat diet (Aguila and Mandarim-De-Lacerda, 2003; Susztak *et al*., 2006; Altunkaynak *et al*., 2008; Deji *et al*., 2009). In the present study, dietary inclusion of ghee (HFD) also resulted in pathological changes in kidney suggesting that dietary lipids can induce damage to kidney tissues. Obesity is a hall mark of high fat diet. High fat diet induced weight gain was reported in many studies (Matsuzawa-Nagata *et al*., 2008; Garcia *et al*., 2018). In our study, dietary inclusion of ghee having high fat content also resulted in significant weight gain after 4-weeks. Female weight gain was more pronounced as compared to males.

Oxidative stress produces reactive oxygen species (ROS) that target many macromolecules of cell including DNA, proteins and lipids. Lipids are the most prominent target of ROS and results in lipids peroxidation (LPO). Elevated LPO has been correlated with diabetes in many reports (Maritim *et al*., 2003; Shamsaldeen *et al*., 2018). Increased level of lipid peroxidation was considered the reason for renal dysfunction in hyperglycemic mice (Oršolić *et al*., 2013). Consistent with these findings, similar increase in oxidative stress was reported in humans with diabetes (Binici *et al*., 2013; Tatsch *et al*., 2015).

Ishii *et al*. (2010) reported that high fat diet leads to hyperglycemia in mice that progress to type-II diabetes. Decrease level of plasma reduced glutathione was recorded in hyperglycemic and diabetic rats (Korkmaz *et al*., 2012; Aydin *et al*., 2019).

Generation of ROS and abnormal levels of anti-oxidants may be responsible for oxidative stress in mice fed with high-fat diets. Changes in the levels of anti-oxidants like catalase, glutathione reductase has been associated with metabolic syndroms like type-2 diabetes (Kumawat *et al*., 2013; Miranda-Díaz *et al*., 2016). Ramesh and Pugalendi (2006) reported that diabetic rats have increased plasma LPO and decreased level of catalase and GSH. Similarly, Aydin *et al*. (2019) reported that hyperglycemic mice has higher LPO and GST activity while decreased level of GSH and catalase in kidney. Echeverría *et al*. (2018) reported that high fat diet resulted in oxidative damage. A significant increase in GST activity was recorded in mice liver fed with HFD which is in accordance with our study.

CONCLUSION

Results of the present study suggest that dietary inclusion of cheap ghee available in the local market resulted in significant weight gain, histopathological changes in kidney and oxidative stress which may be underlying cause of various metabolic changes observed. It is also concluded that both diets have more effect on female as compared to males. It is recommended that long term studies must be undertaken in order to define qualitative and quantitative parameters. Therefore, the effects of high fat diet, locally made ghees and their detrimental effects on human health and their anecdotal effects in local population can be evaluated more precisely.

Statement of conflict of interest

The authors have declared no conflict of interest.

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