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EFFECTS OF USED SUNFLOWER OIL AND GHEE (CLARIFIED BUTTER) ON LIPID PROFILE AND ANTIOXIDANTS IN SD RATS

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ABSTRACT
This study was aimed at determining the effects of used ghee and sunflower oils after potato frying on antioxidant status and lipid profile in SD rats. Male albino SD rats weighing approximately 200-250g were used for the experiments. Left over ghee (FG) and sunflower oil (FS) after potato frying were used to prepare experimental diets. Normal ghee (NG) and normal sunflower oil (NS) was used to prepare the diets for positive control. The study indicated that ghee when administered to rats along the feed maintained the weight, reduced the oxidative stress and had protective effects on liver when compared with Positive Control (PC1) and Experimental (EX1) groups. There was a significant increase in the level of TBARS in EX1 group that received FS, as compared to other groups and the levels were also high in PC1 that received NS. The results on lipid profiles showed a marked and significant elevation in the triglycerides and light density lipoprotein (LDL) and marked decrease in EX1 ($p<0.001$) and PC1 when compared with NC. FS significantly reduced the levels of reduced glutathione in plasma as compared to the levels in NC. Catalase and superoxide dismutase activities were also significantly high in EX1. The study concludes that Ghee is better cooking oil as compared to sunflower oil.

KEYWORDS: Sunflower oil, Ghee, Oxidative stress, Reactive oxygen species, Thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), Lipid peroxidation.

INTRODUCTION
Cooking oil or fat is trimester of fatty acids and glycerol. Various types of cooking oils e.g. soybean oil, cottonseed oil, canola oil, safflower oil, sunflower oil, are at present available and are being used for cooking. These oils are rich in polyunsaturated fatty acid. Omega-3 and Omega-6 fatty acids are very common in cooking oil. These fatty acids are essential fatty acids and should be consumed either in equal amounts or Omega -6 fatty acid should not exceed more than 3 times of Omega-3 fatty acids (Simopoulos, 2011) . Vegetable oils are very high in Omega-6 fatty acid called linoleic acid, which can contribute to all sorts of problems in large amounts. There are reports indicating the high increase in the linoleic acid content of human fat cells and cell membranes in the past few decades (Baylin et al, 2002; Ren et al, 2008). Linoleic acid from vegetable oils increases oxidative stress in the body which is linked to many physiological disorders including endothelial dysfunction leading to cardiovascular diseases (Fang et al, 1996; Turpeinen et al, 1998).

Sunflower oil is one of the most commonly used vegetable oils in the preparation of food at home and also in food manufacturing industries. It is a rich source of Vitamin E, A and D with low saturated fats and has the ability to withstand high temperatures. As per British Pharmacopoeia obtained from Wikipedia, it contains Palmitic acid (saturated): 5%, Stearic acid (saturated): 6%, Oleic acid (monounsaturated omega-9): 30%, Linoleic acid (polyunsaturated omega-6): 59%. Pal et al (2015), reported 95% unsaturated fatty acid in sunflower oil. As the sunflower oil contains vitamin E, it can lead to decreased clotting of blood and this can elevate the risk of bleeding. High omega 6 fatty acid content of sunflower oil could lead to the deleterious effects on health like inflammation and oxidative stress related disorders (Simopoulos, 2006). Sunflower oil has also been reported to induce hyperlipidemia and hyper cholesteremia (Latha et al, 2010).

Ghee, also known as clarified butter, has been used in India as cooking oil and also in Ayurveda for preparation of various medications. In the past several decades, usage of ghee has been a serious concern in terms of a causative factor for the development of coronary artery disease (CAD) due to its content of saturated fatty acids and cholesterol. Latest reports are not consistent with this fact that ghee is one of the causative factors in the development of CAD. According to Chinnadurai et al (2013), it improves lipid profiles by increasing HDL content and reduces lipid peroxidation. Inclusion of ghee

did not increase lipid peroxidation and maintained the levels of serum total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides; decreased liver total cholesterol, triglycerides. Similar results were seen with heated (oxidized) ghee (Sharma et al, 2010). It has been reported to reduce inflammation by reducing serum prostaglandins and leukotriens from peritoneal macrophages in rats (Dwivedi et al, 2002).Ghee because of its high conjugated linoleic acid content, it increases anti-oxidant status and antiatherogenic potency in experimental wistar rats. It also possess anti carcinogenic effects on mammary gland (Rani et al, 2011).

Milk fat contains over 400 individual fatty acids and their isomers. Cow milk contains large amount of saturated short chain fatty acids (72.4%) particularly C14:0 and C16:0, Monounsaturated fatty acid (18.6%) n-6 PUFA and n-3 PUFA in ratio of 2:1 which is very perfect to maintain physiological homeostasis (Kenelly, 1996) As per Ayurvedic literature, ghee is recommended daily for healthy diet because it rejuvenates and promotes longevity, protects the body from various diseases, enhances the digestion and improves the absorption and assimilation (Tirth 1998).

Frying is the most common and traditional way of cooking where the oil is used repeatedly for frying. Repeated heating alters the fatty acid composition because the oil undergoes a series of chemical reactions like polymerization, oxidation and breakdown of triglyceride to fatty acids and glycerol (Choe et al, 2006; Choe et al, 2007). Prolonged heating leads to conversion of glycerol to acrolein which results into the degradation of flavor and nutritional value of the oil. It is a reactive compound and hence it is very toxic. Fatty acids also oxidize very easily and heating accelerates the oxidation process leading to production of peroxidation products like carbonyls, aldehydes, ketones, alcohols and cyclic fatty acids which are toxic to human (Rani et al, 2010). Formation of reactive oxygen species has also been reported during heating process which initiates oxidative chain reaction of biological macromolecules and thus initiates oxidative stress. Thus regular consumption of repeatedly heated oil could be detrimental to health because of its oxidative stress inducing nature. It has also been reported that, there is a positive association between the risk of hypertension and heated cooking oil consumption (Soriguer et al, 2010, Jaarin et al, 2011).

Contradictory reports on ghee and sunflower oil regarding their usage in cooking and their impacts on health issues are still in debate. Therefore the present study has been planned with an aim to determine the effects of multiple heated ghee and sunflower oil after potato frying on antioxidant status and lipid profile of rats fed on these oils along with their food.

MATERIALS AND METHODS

Sunflower oil and Ghee

Sunflower oil of PAN brand and Ghee of Parmalat brand were purchased from Choppies Hyper, in Gaborone, Botswana.

Experimental Animals

Male albino SD rats weighing approximately 200–250 g were used for experiments and were kept in polyethylene cages at an ambient temperature of 25-28ºC with a 12-hour cycle of light and dark. Animals had free excess to water ad libitum and were fed on commercial normal diet for rats bought at Sefalana, a hyper store in Gaborone Botswana. The experiments were conducted following internationally accepted principles for laboratory animal care at the Department of Biology, University of Botswana, Botswana.

Frying procedure and preparation of diet

The oils were heated according to the method described by Owu et al (1998). Two litres of ghee/sunflower oil were heated in steel pot for 5 minutes. A quarter of kilograms of potato chips were then fried at medium flame for 5 minutes. After frying the chips were discarded and ghee and sunflower oil were again used for frying the next day. Frying was repeated for 4 more days. No fresh oil was added between the frying processes to make up for the loss due to frying. The left over ghee (FG) and sunflower oil (FS) were used to prepare experimental diet. Normal ghee (NG) and normal sunflower oil (NS) was used to prepare the diets for positive control groups. The experimental diet was prepared by mixing 100 g of oil with 900g of pounded normal diet pellets. After mixing, the pellets were again prepared by using distilled water and drying in oven at 100^0C.

Chemicals

All the chemicals were of AR Grade and bought from local suppliers. Commercial kits were purchased from Agaape Diagnostic, India.

Biochemical Measurements

Thiobarbituric acid reactive substances (TBARS) in plasma were measured by the method described by Chaturvedi et al, 2007 and reduced glutathione (GSH) was estimated by the method of Ellman, (1959). Method described by Kakkar et al, (1984) was followed to estimate the activities of Superoxide dismutase (SOD) in plasma. Activities of catalase (CAT) in plasma were estimated by the method of Bisswagner, 2004. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), LDL, HDL, triglycerides and total cholesterol were estimated by using the kits from Aggape Diagnostics, India.

Experimental design

The animals were divided into five groups of five rats each and maintained as follows:

Group 1- NC-Normal control- received commercial diet without any oil
Group 2- PC 1-Positive control- received commercial diet supplemented with NS.
Group 3- PC 2- Positive control- received commercial diet supplemented with NG.
Group 4- EX 1-Experimental group- received commercial diet supplemented with FS.
Group 5- EX 2-Experimental- received commercial diet supplemented with FG.

The experiment was run for two months. At the end of the experiment the rats were fasted overnight and the rats were killed with mild ether anesthesia. Blood was collected in heparinized tubes and centrifuged at the rate of 6000 rotations per minute. Plasma samples were drawn and stored at -70ºC for various biochemical estimations. Weight of all the animals was taken in the beginning and also at the end of experiment.

Statistical Analysis

All data were expressed as mean±SEM. Analysis of variance was performed by one way ANOVA and subjected to Tukey's test for multiple comparisons. P-value ≤ 0.05 was considered statistically significant value. In all these cases, Statistical Software Sigma stat, 3.1 was used to for analysis.

RESULTS

Percent Increase in Weight

The average weights of these animals are shown in Figs. 1 which shows a gradual increase in weight from week one onwards in PC1 and EX1. Gradual deposition of fat in the adipose tissue might be the cause of gradual weight gain. A significant increase in the weight was noted in PC1 and EX1 when compared with NC, PC2 and EX2 ($p<0.01$ in all the cases). There was no significant difference in weight gain of NC. Rats in group PC2 and EX2 showed significant reduction in weight.

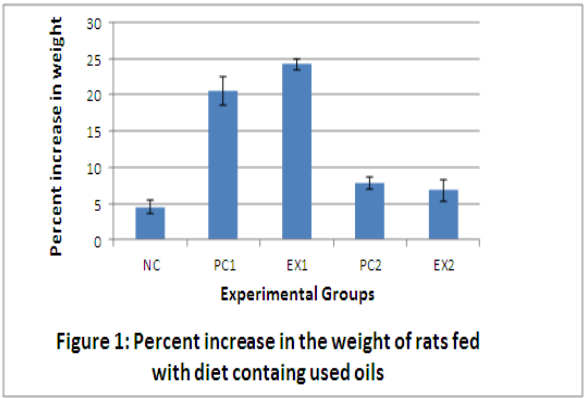

Figure 1: Percent increase in the weight of rats fed with diet containg used oils

Plasma Lipid Peroxidation and liver function indices

The results presented in Table 1 shows the effects of sunflower oil and ghee on plasma thiobarbituric (TBARS) acid and liver function indices; alanine aminotransferases (ALT), aminotransferases (AST), alkaline phosphatases (ALP) concentrations in plasma serum after 2 months of feeding used cooking oil contained food. There was a significant increase ($P<0.001$) in the level of TBARS in EX1 group that received FS, as compared to other groups. Levels were also high in PC1 that received NS ($p<0.05$) as compared to NC. A non- significant increase was also noted in EX2. Transferases also showed similar trends. Activities of these enzymes were very high in in EX1 followed by PC1. Activities were very close to NC in PC2 and EX2 group.

Table 1: Effects of fresh and used ghee and sunflower oil marker parameters.

Parameters / Groups	TBARS (µmoles/L	AST (U/L)	ALT (U/L)	ALP (U/L)
NC	1.15±0.06	41.40±1.26	40.64±1.03	43.80±3.12
PC1	1.82±0.17*	54.13±2.89	42.40±3.06	47.98 ± 1.74
EX1	4.25±0.57***	84.25±1.94**	82.80± 3.66**	89.04±3.04**
PC2	1.30±0.13	41.87±3.86	44.20±2.85	41.45± 3.16
EX2	1.44± 0.11	46.65±1.72	53.40±2.68	53.61± 2.16

*p<0.05, ** p <0.01, *** p<0.001 when compared among control and experimental groups

Lipid Profiles

The result presented in Table 2 shows the effects of oil and ghee on plasma lipid profile. There was a marked and significant elevation in the level of triglycerides in EX1 ($p<0.001$) when compared with NC. Level in PC1 also showed significant elevation in the level ($p<0.001$). No significant increase was noted in EX2 and PC2. Similar trend was shown by LDL cholesterol. In group PC2 and EX2, levels were low, but still differed significantly when compared with NC ($p<0.01$). In case of HDL opposite trends were noted. The HDL level was markedly reduced in EX1 and PC1. In the group EX2 and PC2 also, the reduction was noted but it was non-significant when compared with NC.

Table 2: Effects of fresh and used ghee and sunflower oil on Lipid Profile.

Parameters / Groups	Triglycerides (mg/dl)	Total Cholesterol Mg/dl	HDL (mg/dl)	LDL (mg/dl)
NC	91.98±1.57	58.80±4.26	54.40±2.96	13.67±3.26
PC1	162.21±4.36***	83.60±4.22**	39.45±1.80*	41.60±4.31***

EX1	232.40±10.21***	113.28±4.01***	27.52±2.26**	64.60±6.45***
PC2	93.94±4.70	65.58±5.56	51.93±2.10	22.28±2.03**
EX2	113.61±4.30	70.64±3.03*	48.92±1.86	25.29±1.99**

*p<0.05, ** p <0.01, *** p<0.001 when compared among control and experimental groups.

Anti-oxidants

Effects of ghee and sunflower oil on antioxidants levels are presented in Table 3. It shows that FS significantly reduced the levels of reduced glutathione and CAT in EX1 as compared to NC (p<0.01). On the other hand, FS enhanced the activities of SOD in EX1 as compared to the activities in NC. Similar effects were also observed for NS in PC1 but it had less significant effect than FS. NG and FG had maintained the levels of reduced glutathione in EX2 and PC2 and also the activities of CAT and SOD in both the groups.

Table 3: Effects of fresh and used ghee and sunflower oil on antioxidants.

Parameters Groups	Reduced Glutathione (mg/dl)	Catalase (U/mg Hb)	Super-Oxide Dismutase (U/mg Hb)
NC	51.80±5.22	42.85±1.99	2.77±0.26
PC1	37.87±2.37**	38.57±2.96*	4.66±0.32**
EX1	33.80±3.56**	32.48±4.70**	5.53±0.54**
PC2	47.24 ±1.72	44.94±2.21	3.05±0.37
EX2	40.42±2.52	48.02±2.42	2.73±0.22

*p<0.05, ** p <0.01when compared among control and experimental groups

DISCUSSION

It is very clear from the present study that ghee (the melted and fried one) when administered to rats along the feed reduces the weight, oxidative stress and having protective effects on liver as compared to NC, PC1 and EX1. The reduction was very much pronounced and significant in PC2. On the contrary rats in group EX1 that were fed the FS and in group PC1 fed normal sunflower oil, showed significant increase in percent weight compared to NC and other groups. Rats in these groups also showed extreme conditions of oxidative stress concluding to liver toxicity and dyslipidemia.

Reduction in the weight in PC2 and EX2 might be due to the high content (70%) of saturated fat content of ghee which is made mainly of short to medium chain fatty acids. Short to medium chain triglycerides are digested directly into the blood stream without being packaged into lipoproteins and are then transported to the liver. As they don't need bile or pancreatic enzymes to break down, they are easier to digest. Because of their short carbon chain, they are of low calorific value and provide quick energy, like carbohydrates. This is one way of contributing weight loss and preventing obesity. Recently it has been reported that short chain fatty acids protect against obesity via a PPARγ-dependent switch from lipogenesis to fat oxidation (Besten Gd, 2015). Conjugated linoleic acid of ghee could also be one of the reasons for weight loss as it reduces visceral and ectopic lipid accumulation in rats (Malinska, 2015). Sun flower oil is rich in n-6 PUFA which is easily oxidized generating oxidative stress which is one of the causative factors linked to obesity. Another significant cause of obesity is the hydrolysis of ARA producing 2-arachiodonoylglycerol (2-AG), which is a predominant ligand of cannabinoid receptors of brain that stimulates food intake and lipogenesis crowning to obesity (Di Marzo et al,2005, Ailhaud et al, 2006].

Rats that consumed FS were also under oxidative stress and results of lipid profile showed dyslipidemic conditions. Oxidative stress leads to lipid peroxidation and membrane damage. This is the reason of elevated activities of ALP, AST and ALT. These are the enzymes localized in hepatocytes and are released in circulation indicating liver damage. Oxidative stress and obesity are always associated with dyslipidemia. Rats given FS were under severe oxidative stress, indicated by high levels of TBA in plasma accompanied by reduced GSH level and enhanced activities of catalase and SOD, as compared to other groups followed by the group fed on normal sunflower oil. Rats that received NG, showed no oxidative stress. Sunflower oil is rich in PUFAs that contribute enormously to oxidative stress in various ways. PUFAs, especially ARA and LA, are primary targets for free radical and singlet oxygen oxidations that results into oxidative stress (Jarrin et al, 2012, Vigor 2014). Sunflower oil is rich in Vitamin E but has high content of PUFA that oxidizes very easily even at room temperature. Frying process accelerates oxidation, hydrolysis and polymerization of fatty acids and produces variety of free radicals like hydroperoxides and aldehydes which are absorbed into the food. The process is also enhanced because of destruction of anti- oxidants like vitamin E present in the sunflower oil at high temperature (Marinova 2012). Consumption of such oil loaded with free radicals generates oxidative stress and concludes into various physiological disorders. Prolonged oxidative stress causes development of impaired insulin action that enhances the over production of triglyceride rich lipoprotein, very low density lipoprotein (VLDL) and

its reduced clearance due to reduced activity of lipoprotein lipase, a rate limiting enzyme responsible for clearing the triglycerides rich lipoprotein, the VLDL (Semenkovich, 2006). These processes cause the elevation of circulating triglycerides. Prolonged stay of VLDL in circulation, results in exchange of triglycerides in VLDL for cholesterol esters in high density lipoprotein (HDL). These HDL which are rich in triglycerides are readily hydrolysed by hepatic lipase resulting in the fast clearance of HDL from the circulation (Taskineen, 2003).

On the other hand, group received normal ghee did not show any oxidative stress. Rats received fried ghee showed the oxidative stress but it was non-significant when compared with NC. These results are consistent with the previous report by Sharma et al (2010). Ghee contains saturated fatty acids (70%) which are not easily oxidizable. Non -significant elevation in the activities of catalase and SOD and in the level of reduced glutathione in group received FG could be due to destruction of contained anti-oxidants; Vitamin A, D and carotene. Apart from this, ghee contains decosahexanoic acid and conjugated linoleic acid which are responsible to reduce the oxidative stress, weight and dyslipidemia.

In conclusion, the results indicated that the frying process accelerates oxidation, hydrolysis and polymerization of fatty acids and produces variety of free radicals. Fried sunflower and normal sunflower promoted weight gain and lead to oxidative stress which concluded to liver toxicity and dyslipidemia. Normal ghee did not produce any of these effects. Non-significant effects were noted in case of fried ghee. Thus it can be concluded that ghee is the better cooking oil as compared to sunflower oil.

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