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Effects of oxidized vanaspati ghee on the serum lipids profile and radical scavenging activity of the in vitro lipids of liver, brain and muscles

[Okside vanaspati ghee yağının serum lipid profili ve karaciğer, beyin ve kas lipidlerinin radikal temizleyici aktivitesi üzerine in vitro etkisi]*

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ABSTRACT

Objectives: The aim of the study is to determine the effects of thermal oxidation of vanaspati ghee on the serum lipids profile and radical scavenging activity of the experimental animals.

Methods: Vanaspati Ghee was thermally oxidized at 130°C for 9, 18, 27, 36 and 45 h respectively. The oxidized ghee was fed to the rabbits. The serum lipids profile was determined using standard protocols and kits. Radical scavenging activity was determined with the help of 2,2-diphenyl-1-picrylhydrazyl free radicals.

Results: It was found that oxidation increase formation of hydroperoxides and decrease the level of radical scavenging activity. Thermally oxidized ghee was supplied to the rabbits for one week. The serum lipid profiles showed a dose dependent increase in triglyceride, total cholesterol and LDL-cholesterol. No statistically significant increase was observed in the HDL-cholesterol with increase of oxidation time. Glucose and body weight decreases significantly and was highly correlated to the serum lipids profile. Radical scavenging assay of the lipids extracted from the liver, brain and muscles tissues decreases significantly.

Conclusion: These results showed that thermal oxidation produced oxidized compounds and the amount of oxidation increase with increase of treatment time. The oxidized ghee was producing significant effects in the serum lipids profile as well as peroxidation in different tissue. Therefore oxidation for longer time should be avoided.

Key Words: Vanaspati ghee, thermal oxidation, rabbit, serum lipids profile, radical scavenging activity

Conflict of Interest: There is no conflict of interest among the authors to the present study, as well as to any person or institution or agency.

ÖZET

Amaç: Çalışmanın amacı termal olarak okside edilmiş olan vanaspati ghee yağının serum lipid profili ve deney hayvanlarında radikal temizleyici aktivite üzerine olan etkisini belirlemektir.

Yöntem: Vanaspati ghee yağı termal olarak 130°C'de 9, 18, 27, 36 ve 45 saat tutularak oksitlenmiştir. Tavşanlar okside edilmiş olan yağ ile beslenmişlerdir. Serum lipid profili standart yöntemler ve kitler kullanılarak tayin edilmiştir. Radikal temizleyici aktivite 2,2-difenil-1-pikrilhidrazil serbest radikallerinin yardımıyla belirlenmiştir.

Bulgular: Oksidayonun hidroperoksit oluşumunu arttırdığı ve radikal temizleyici aktiviteyi düşürdüğü bulunmuştur. Termal olarak oksitlenen yağ tavşanlara bir hafta boyunca verilmiştir. Trigliserid, total kolesterol ve LDL-kolesterolü içeren serum lipid profilinde doza bağlı olarak artış gözlenmiştir. HDL-kolesterol ile oksidasyon zamanı arasında istatistiksel olarak anlamlı bir fark bulunmamıştır. Glukoz ve vücut ağırlığı serum lipid profile ile ilişkili olarak belirgin düşüş göstermiştir. Karaciğer, beyin ve kas dokularından ekstrakte edilen lipidlerin radikal temizleyici aktivitesi belirgin şeklilde azalmıştır.

Sonuç: Bulgular termal oksidasyonun okside bileşikler ürettiğini ve oksidasyon miktarının muamele süresine parallel olarak arttığını göstermektedir. Okside yağ serum lipid profilini ve farklı dokulardaki peroksidasyonu belirgin bir şekilde etkilemektedir. Bu nedenle uzun süreli oksidasyondan kaçınılmalıdır.

Anahtar Kelimeler: Vanaspati ghee yağı, termal oksidasyon, tavşan, serum lipid profili, radikal temizleyici aktivite

Çıkar Çatışması: Yazarlar arasında çıkar çatışması bulunmamaktadır.

Introduction

Vegetable oils and fats are main components of our diet. The major composition of these lipids is the triacylglycerols, small amount of cholesterol, and various natural antioxidants. During cooking or frying of foods, these triacylglycerols and cholesterols formed oxidized compounds like hydroperoxides, epoxides, hydroxide [1]. These oxidized compounds produce significant changes in the composition and properties of fats or oils. For example Bectar and Narayanan showed that development of peroxides and epoxides took place and increased during heating of ghee at various temperatures [2]. The hydroperoxides are usually oxidized or degraded to a variety of oxidation products. During cooking or frying of foods, these oxidized compounds are absorbed into the foods matrix and thus enters human body. It was established that oxidized fats and oils are toxic [3]. The oxidized fats are significance to human health, because some of these oxidation products may be carcinogenic [4-5]. These oxidized compounds produced in the dietary oxidized oils were also found to enhance the growth of hepato-carcinoma [6]. Thus oxidation and formation of oxidation compounds in oils or fats is hot topic from the industries to the consumer.

Vanaspati ghee is a vegetable oil-based product, widely used in most of the Southeast Asia, and Middle Eastern countries for cooking and frying of foods and vegetables. In certain countries, vegetable ghee may be formulated for general purpose applications, cooking, frying, and baking [7]. Vanaspati ghee is a complex lipid consists of triacylglycerols about 98% of the total material. Bhanger and Farooq studied the fatty acid (FA) composition and contents of *trans*-unsaturated FA (TFA) in 34 vanaspati ghee, 11 shortenings and 11 margarine samples from Pakistan [8]. The authors showed that vanaspati ghee contains 27.8–49.5, 22.2–27.5, 9.3–13.1%; vegetable shortenings 37.1–55.5, 15.8–36.0, 2.7–7.0%; and margarines 44.2–55.8, 21.7–39.9, 2.9–20.5%, of saturated FA, cis monounsaturated FA, and cis polyunsaturated fatty acids (PUFA), respectively. These authors also showed higher amounts (14.2 to 34.3%) of TFA. Ghee also contains small amount (0.15 to 0.31%) cholesterol. Vegetable ghee is also oxidized by heating [9], as well as fluorescent light [10]. However there is lack of literature regarding the effects of thermally oxidized vanaspati ghee on the lipid profile. To the best of our knowledge this paper presents the prime data on the effects of thermally oxidized vanaspati ghee on the serum lipids and radical scavenging potential of the lipids from the liver, brain and muscles of the experimental animals.

Materials and Methods

Materials

Fresh vanaspati ghee was obtained from the local market. Glucose and cholesterol was from Sigma Aldrich (Germany). All other chemicals and reagents were of American Chemical Society (ACS) grade from Sigma Aldrich USA or otherwise mentioned.

Thermal oxidation

Vanaspati Ghee was thermally oxidized on hot plat at 130 ± 5°C, for five consecutive days for 9 h. At the end of each day, a representative ghee sample was taken and stored in refrigerator at -20°C. Thus the ghee sample was oxidized for 9, 18, 27, 36 and 45 corresponding to each day of oxidation.

Peroxide value

The peroxide value (POV) of control and oxidized ghee samples were determined using the American Oil Chemists' Society (AOCS) official method (method Cd 8b-90) and expressed as meq O_2/kg of fat [11]. All samples were measured in triplicate or otherwise mentioned.

Experimental animals

Rabbits of the local Himalayan strain were selected for the study because of the highly selective and developed organ system and were easily available animals for these experiments. Rabbits were grouped by random distribution into five groups and each group has 9 animals irrespective of the gender. They were placed in same approved animal house facility and fed fresh diets daily. The rabbits had free access to food and water throughout the study. Before starting the treatments, all animals were placed one week ahead to get familiarized with the environment.

Animal feeding

Rabbits were classified into five treatment groups i.e. 9, 18, 27, 36 and 45 h, which designate the individual oxidized ghee samples. Each single treated group was further classified into three sub-groups designated as A, B and C, which corresponds to 0.5, 1.0 and 1.5 g/ body wt of daily doses of the oxidized vegetable ghee. Rabbits were fed for one week and two days later they were slaughtered. The blood samples were collected in falcon tubes and centrifuge for 10 min to obtain serum for further analysis.

Weight change

The change in the weight of whole rabbit was measured with digital platform scale analytical balance (Marino, China) with 30 kg capacity of weighing.

Extraction of lipids

The tissues of each individual rabbit were taken and stored in formalin. Lipids have been extracted from the selected tissues (liver, brain & muscles) according to the procedure of Folch et al. with little modification [12]. Briefly after homogenization of tissues, chloroform-methanol (2:1) mixture was added. The sample was kept on shaker for 98 h at 30 rpm. To the extracted mixture 10% KCl were added, washed and filtered. The solvent was evaporated using rotary evaporator.

Analytical parameters

Biochemical parameters like cholesterol, HDL-cholesterol, LDL-cholesterol and glucose were

measured using HUMAN (Germany) kits, while for total triglycerides DiaSys (Germany) kit was used. The quantification was carried out using six point standard calibration curves with help of Shimadzu UVvis-1700 spectrophotometer (Shimadzu, Japan).

Radical scavenging assay

Radical scavenging assay (RSA) of the oxidized ghee, serum and lipids samples extracted from the tissues according to the procedure of Lee et al. with some modifications [13]. Briefly, an ethyl acetate solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was freshly prepared at a concentration of 0.1 mM. Five milliliters of 0.10 mM DPPH solution were mixed with 56 µL oil samples in a 30 mL serum bottle. After 30 min in dark, the absorbance of the sample mixture was measured at 515 nm using a UVvis-1700 spectrophotometer (Shimadzu, Japan). The RSA toward DPPH radicals was estimated from the differences in absorbance of the DPPH solution with or without sample (control), and the percentage of RSA was calculated from the following equation:

Radical scavenging activity (%) = (Ac-As/Ac) × 100

Statistical analysis

All samples were measured in triplicate or otherwise mentioned. Data were analyzed by one-way analysis of variance (ANOVA) and Holm-Sidak method of multiple comparison method at $\alpha = 0.05$ using SigmaPlot for windows version 11.0 (Systat Software, Inc, 2008).

Results and Discussion

Deep fat frying is a traditional method of cooking and is popular throughout the world for preparation and manufacture of nearly all foods [14]. In restaurants, fast food chains as well as in industrial frying operations such as potato chips, instant noodles, fat frying is the main procedure. In Pakistan, deep ghee processed food products are one of the key choice of the consumer from the street foods to the restaurants due to high price of vegetable oils. During frying, the ghee is exposed continuously or repeatedly to elevated temperatures in the presence of air, moisture and foods. The oxidized ghee or oils thus enters the food matrix and produce toxic effects [1]. However due to the absence of literature and knowledge, these foods are becoming one of the most serious health risk factor of the country as well as across the ghee using countries. This study will not completely diagnose the relation of risk, but may serve the preliminary source of knowledge in this regard.

Hydroperoxides contents

Fatty acids or triacylglycerols are oxidized by thermal oxidation to form hydroperoxide. These hydroperoxides are the further oxidized or degraded to secondary oxidation products of various chain lengths (1, 15). Peroxide value is the measure of the hydroperoxides formed in fats and oils. Figure 1A shows that peroxide value increase with increase of oxidation time with respect to control. The oxidation was statistically significant at $P < 0.001$. The results are in agreement with Zeb & Murkovic who showed that peroxide values increases with increase of oxidation time [15]. Similarly Ahmad et al. showed that peroxide value of vegetable ghee increases with increase of irradiation doses and fluorescent light respectively [9-10]. This means that thermal stress as well irradiations of vegetable ghee results enhance the formation of hydroperoxides. These hydroperoxides produces secondary oxidation compounds and thus the quality of the vanaspati ghee decreases and eventually not acceptable to the consumer.

Radical scavenging assay of vanaspati ghee

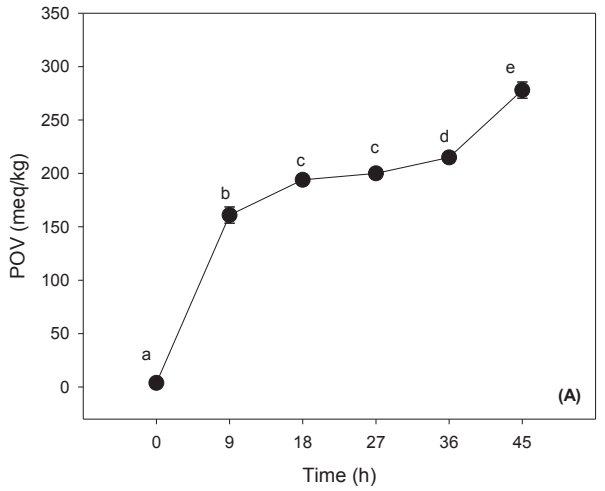
The vanaspati ghee manufacturers usually add synthetic vitamin A and D, because during the ghee manufacture a significant loss of these natural antioxidants occurs. These synthetic antioxidants are important in nutritional as well provide protection against any possible stress during storage or cooking [13]. The radical scavenging assay is therefore more reasonable parameter to determine the total antioxidant potential of the ghee. The radical scavenging assay of the control (58.0%) and oxidized vanaspati ghee shows a significant decrease and reached a value of 16.3%. This shows that thermal oxidation release significant amount of free radicals and loss of antioxidant potential. Figure 1B shows that %RSA value was highly correlated (R^2=0.9445) with the increase of hydroperoxides contents. The increase of hydroperoxides thus decreases the radical scavenging activity.

Effects on body weight

Growth is an important parameter for studying the effect of a specific diet. Figure 2A showed a significant loss of the body weight with increase of oxidation time. The loss was statistically significant at $P < 0.05$ in all three subgroups. Previously it was observed that vanaspati ghee fed to the rat showed increase of weight [16]. The present results are the clear picture of the involvement of oxidized ghee in the retention of growth rate and also loss of the body weight. The main reason may be the oxidation of proteins as well as the disturbance of the metabolism of the lipids in the body. This was confirmed by studying the correlation of weight change, triglyceride and oxidation time as shown in the Figure 2B. It has been observed that increasing oxidation time is highly correlated with changes in the weight and serum triglyceride contents. These oxidized lipids compounds are contributing to the enhancement of the free radical reactions in the body and thus changing the body weight.

Effects on serum lipids

The effects of oxidized vanaspati ghee on the serum lipids like triglycerides, cholesterol, and high and low density lipoproteins were the special interest of this work. Table 1 show the effects of thermally oxidized vanaspati ghee on the serum lipids profile of the rabbits. It has been observed that at lower feeding dose of A, the triglyceride concentrations increases significantly with



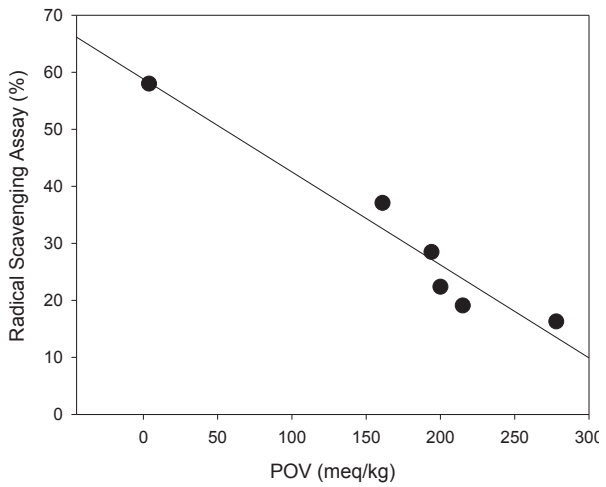
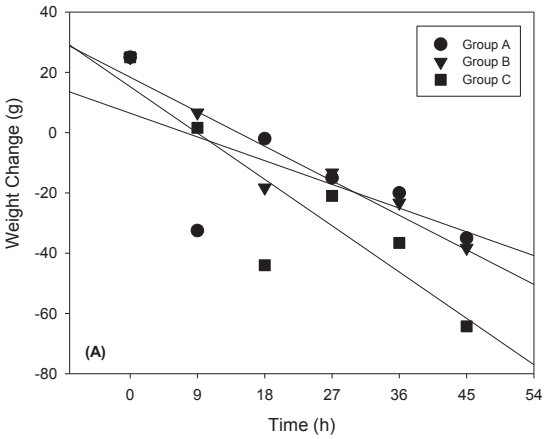


Figure 1. Characteristics of the thermally oxidized vanaspati ghee, (A) Peroxide value (meq/kg) of the vanaspati ghee, and (B) Correlation of radical scavenging activity (%) and peroxide value (POV) of the oxidized vanaspati ghee. Figure A shows a time dependent increase of peroxide value, while Figure B shows that decrease in the radical scavenging is highly correlated with the increase in peroxide value.



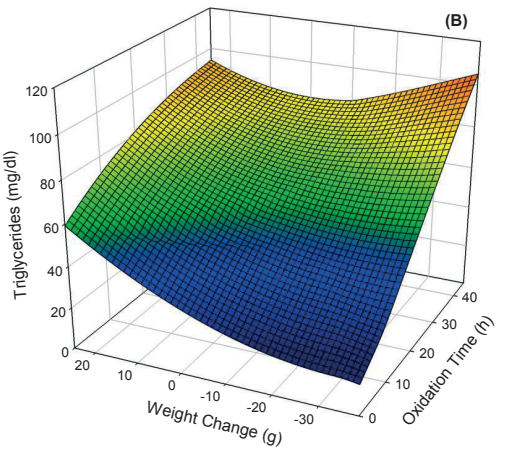


Figure 2. Effects of oxidized vanaspati ghee, (A) Effects of change in the body weight, and (B) Correlation of triglyceride, body weight change and oxidation time. There are significant changes in the body weight of rabbits with increase feeding of oxidized samples (Figure A). Figure B shows that the triglycerides are highly correlated with the change in the body weight as well as oxidation time.

Table 1. Radical scavenging assay (RSA) of the lipids extracted from the liver, brain and muscles of the rabbit.

Sample	Dose	Concentration (mg %)					
		Oxidation Time (h)					
		Control	9	18	27	36	45
Triglyceride	A	59.2 ± 3.1	52.3 ± 6.1	71.6 ± 7.9	74.0 ± 5.7	61.2 ± 2.3	95.3 ± 7.2
	B		49.0 ± 2.1	49.7 ± 3.0	64.0 ± 8.2	79.8 ± 9.1	105.2 ± 1.2
	C		43.2 ± 4.5	50.3 ± 5.0	63.8 ± 5.2	110.3 ± 7.6	79.9 ± 3.1
Total Cholesterol	A	66.6 ± 4.1	116.7 ± 7.1	88.9 ± 14.2	125.6 ± 7.4	92.3 ± 12.1	125.0 ± 7.0
	B		111.0 ± 11.1	128.0 ± 10.2	116.4 ± 11.2	99.8 ± 17.2	107.3 ± 7.4
	C		84.5 ± 6.5	97.3 ± 6.3	90.6 ± 16.2	85.9 ± 7.5	101.2 ± 18.2
HDL-cholesterol	A	45.4 ± 2.3	67.2 ± 1.2	67.0 ± 0.5	50.2 ± 6.1	69.3 ± 3.7	65.5 ± 0.9
	B		68.1 ± 1.0	65.2 ± 1.1	66.5 ± 0.9	65.4 ± 1.3	62.2 ± 1.3
	C		56.2 ± 3.1	45.5 ± 4.3	50.2 ± 6.4	58.4 ± 2.1	61.4 ± 2.3
LDL-cholesterol	A	21.5 ± 2.1	48.2 ± 6.9	29.3 ± 5.2	59.2 ± 7.1	37.3 ± 2.1	59.4 ±7.2
	B		43.2 ± 9.7	63.2 ± 10.1	49.6 ± 11.1	43.6 ± 2.1	44.5 ± 7.3
	C		28.4 ± 12.1	53.5 ± 4.3	50.2 ± 7.1	26.4 ± 5.5	48.5 ± 4.2

Values are the means of triplicate (n=3) readings.

relative value of 12%, 14%, and 36% at 18, 27 and 45 h, while no significant change was observed at 9 and 36 h. While at higher doses such as B & C, the amount was initially decreases (10 to 16%) and then a significant increase (about 46 and 20%) occur with the increase of oxidation time. Kumar et al. showed that feeding heated desi ghee prepared from milk significantly lowered the triglycerides contents of the experimental animals [17]. This is due to the difference in the sources of ghee (animal & plant), oxidation level, and animals.

Cholesterol is the component of cell membrane and source of vitamin D. The total cholesterol contents increase significantly ($P < 0.05$) at the doses of A & B with increasing oxidation time. While in the case of dose C, it was increased by 18% initially and reached an increase of 35% on 45 h sample. In the case of HDL-cholesterol there were increase in the total amount, but the increase was not significant in all three doses. While in the case of LDL-cholesterol there was significant ($P < 0.05$) increase due to the increase of oxidation time. Figure 3 shows that the hydroperoxides are highly correlated with the LDL-cholesterol and HDL-cholesterol. The increase of LDL-cholesterol was positively correlated with hydroperoxides. The present results for HDL-cholesterol is in accordance with the Kumar et al. who showed that feeding heated ghee at 5% level produce no significant change in the HDL-cholesterol, while LDL-cholesterol was dropped by 32%, which is inverse to our findings [17]. The main reason may be the presence of trans-fatty acids in the vanaspati ghee [8]. The foods are usually roasted in vanaspati ghee in most of the cases in Pakistan, is thus causing increase in the fatty substances and obesity. Recently vanaspati ghee was found to increase LDL-cholesterol and thus consider harmful [18].

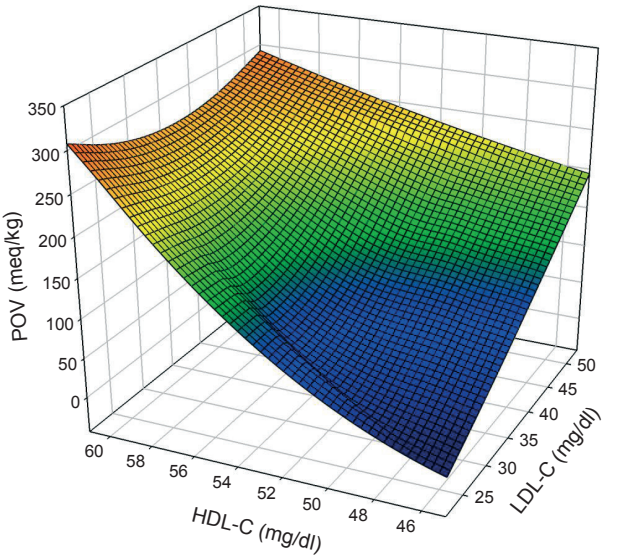


Figure 3. Correlation of peroxide value (POV) of oxidized ghee, serum HDL-cholesterol and LDL-cholesterol values. The increase in the POV values is highly correlated with the change in the serum HDL-cholesterol as well as LDL-cholesterol.

Effects on glucose content

Since glucose is one of important carbohydrate highly linked with the metabolism of lipids. During glycolysis glucose is converted to pyruvate. The decarboxylation of pyruvate produces acetyl CoA. This compounds has many possible pathways, the most important is the conversion to fatty acids, eventually triglycerides, cholesterol and many more lipids substances [19]. Thus it is essential to determine glucose content during the study of *in-vivo* lipid metabolism or lipid related species. Figure 4A shows serum glucose concentration was significantly negative correlated with the increase of oxidation time. The lower glucose concentration can be one of the causes of increase in the contents of triaglycerides, cholesterol, and LDL-cholesterol. Figure 4B shows that decrease in glucose is highly correlated (R^2=0.8827) with the change in the body weight.

Effects on radical scavenging activity of lipids

Free radicals and other reactive oxygen containing compounds are usually generated by exogenous chemicals or endogenous metabolic processes in food systems or in the human body. These radicals may cause oxidative damage and consequently plays a

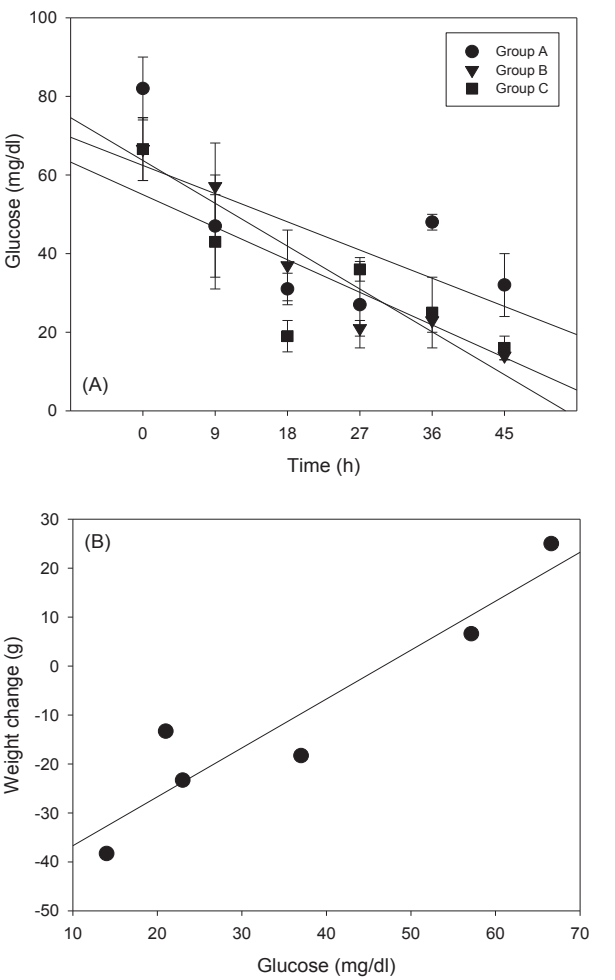


Figure 4. Effects of the thermally oxidized vanaspati ghee, (A) Effects on serum glucose (mg/dl) of the rabbit, and (B) Correlation of serum glucose (mg/dl) and weight change of the rabbit induced by oxidized vanaspati ghee. Decrease in the body weight is highly dependent on the decrease in the serum glucose content.

significant pathological role in some chronic diseases like atherosclerosis, cancer and arthritis [20-21]. In order to overcome this, ingestion of foods containing antioxidants such as ascorbic acid, tocopherols and carotenoids may reduce the oxidative damage. However, thermal oxidation was found to degrade and destroy these antioxidants [22-24], thus decreasing the radical scavenging activity. Table 2 shows the effects of thermally oxidized vanaspati ghee on the radical scavenging activity assay of the lipids extracted from tissues of the liver, brain and leg muscles. Significant reduction in the RSA value has been observed in the liver tissue with increase of oxidation time from 9 h to the 45 h. In the case of brain and muscles tissues, a small initial reduction of about 2-6% and 3-6% respectively in the RSA was observed. Further decrease was observed in both cases with increase of oxidation time. The results showed that oxidized lipids reached liver easily and affect its antioxidant potential than brain and muscle. This also shows that the decrease occurs in the antioxidants level or the increase of reactive species in these tissues may be the main cause of reduction in the RSA values. The reduction in RSA is therefore one of the alarming point.

Conclusion

The effects of thermally oxidized vanaspati ghee on the serum lipid profile and antioxidant activity of the lipids extracted from the different tissues were observed. Ghee was thermally oxidized for 9 to 45 h. It was found that thermal oxidation increase the formation of hydroperoxides and decrease the level of radical scavenging activity. Thermally oxidized ghee was given to the rabbits for one week. There were significant changes in the lipids profile of the animals. Triglycerides, cholesterol and LDL-cholesterol were increased with increasing oxidation time. No statistically significant changes were observed in the HDL-cholesterol with increase of oxidation time. Glucose and body weight decreases significantly and was highly correlated to the serum lipids profile. Radical scavenging assay of the lipids extracted from the liver, brain and muscles tissues decreases significantly. These results showed that thermal oxidation and use of thermally oxidized vanaspati ghee are harmful and it is suggested that longer oxidation should be avoided.

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Table 2. Radical scavenging assay (RSA) of the lipids extracted from the liver, brain and muscles of the rabbit.

Sample	Dose	Radical Scavenging Assay (%)					
		Oxidation Time (h)					
		Control	9	18	27	36	45
Liver	A	42.7 ^{a}	33.8 ^{b}	33.1 ^{b}	30.2 ^{c}	30.1 ^{c}	21.9 ^{d}
	B		35.5 ^{b}	29.8 ^{c}	28.0 ^{d}	26.9 ^{d}	19.5 ^{e}
	C		36.7 ^{b}	32.5 ^{c}	29.5 ^{d}	23.6 ^{e}	15.7 ^{f}
Brain	A	44.4 ^{a}	38.1 ^{b}	34.6 ^{c}	32.7 ^{d}	32.5 ^{d}	22.2 ^{e}
	B		39.0 ^{b}	36.7 ^{c}	29.7 ^{d}	29.8 ^{d}	16.9 ^{e}
	C		42.0 ^{a}	37.9 ^{b}	32.1 ^{c}	25.5 ^{d}	19.6 ^{e}
Muscles	A	45.8 ^{a}	42.9 ^{a}	35.6 ^{b}	34.8 ^{b}	28.8 ^{c}	26.4 ^{d}
	B		41.7 ^{a}	38.3 ^{b}	32.4 ^{c}	28.5 ^{d}	25.7 ^{e}
	C		39.3 ^{b}	39.9 ^{b}	35.9 ^{c}	25.2 ^{d}	23.9 ^{e}

Different letters (*a-f*) in the row represent significance at $p < 0.05$.

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