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Protective Effect of Sweet Potato Peel against Oxidative Stress in Hyperlipidemic Albino Rats

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Abstract

In the present investigation, the valorization of sweet potato (*Ipomoea batatas* L.) wastes into food application with health benefits was studied. Growing interest in the replacement of synthetic food antioxidants by natural ones has fostered research on vegetable sources and screening of raw materials to identify new antioxidants. Different parts of sweet potato contain various phenolic activities. The sweet potato peel had the highest antioxidant activity as compared to other parts. It was reported that sweet potato contains bioactive compounds such as Flavonoids, Polyphenols, The present study was executed to examine the antioxidant components of sweet potato peel and their addition to fermented cow's milk to improve lipid profile in rats fed high fat diet. Fermented milk was prepared from cow's milk fortified with 1% and 2% of sweet potato peels (SPP), before inoculation with starter culture containing (*S. thermophilus* TH4, *L. acidophilus* La5 and *L. bulgaricus*). It was found that addition of SPP had no significant effects on the sensory attributes (appearance, body, texture, and flavor) as compared to the control samples. Increasing the percentage of SPP resulted in a decrease in the viscosity of the fermented milk. When SPP powder was included in the diet and fed to rats, there was a significant decrease in the activities of serum transaminases (ALT and AST). Results also showed that the fermented milk administration to hyperlipidemic/hyper-cholesterolemic rats induced a significant decrease in the total cholesterol, LDL, protein and triglycerides, and a significant increase in HDL concentration. Histopathological examination showed that groups fed with SPP blended fermented milk showed less pathological changes compared to the positive control group. The main results indicated that there is a high potential for sweet potato peel extract to be utilized as antioxidant in food systems due to its high phenolic contents. It can be concluded that the

antioxidant components in purple sweet potato peel when administered with fermented cow's milk can improve serum lipid status in rats fed high fat diet.

Keywords

Sweet Potato Peel, Total Phenolic, Organoleptic Properties, Oxidative Stress, Fermented Milk

1. Introduction

Yogurt is traditionally consumed as a healthy food due to its high nutritional value and health benefits as well as its sensory properties [1]. Yogurt is a rich source of bioactive peptides with antioxidant activity that are produced during fermentation [2]. There is an increasing interest in applying fruit processing wastes as functional food ingredients since they are rich source of dietary fiber, and most of the beneficial bioactive compounds are found to remain in those by-products [3] [4].

The increased consumption of fruits and vegetables is an effective strategy to increase antioxidant intake and to reduce oxidative stress and it may lead to lower risk of developing chronic diseases, such as cancer and cardiovascular diseases [5]. Antioxidant activity of foods is important both for shelf life of the product and protection from oxidative damage in the human body. Phenolics are found in plenty of plants and consist of an aromatic ring within the molecular structure [6].

Food industry generates large amount of wastes or by-products annually around the world from various sources. Food wastes or by-products are an excellent source of nutraceuticals, bioactive, inherently functional and possess many components that are good for human health [7]. Various studies reported that different kinds of waste such as pomegranate peels, lemon peels and green walnut husks can be used as natural antimicrobials [8]. Agri-food industry generates, during the stages of raw material collection and preparation of derived food products, thousands of tons of byproducts, such skins, peels, seeds, leaves, bones, other inedible fractions, or parts discarded due to sensorial characteristics. For a long time, food byproducts have been used for low value-added activities [9].

The high concentration of phenolic compounds present in peels, skins and seeds, supports the utilization of these residues as a source of natural antioxidants. Phenolic compounds exhibit a wide range of physiological properties such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thermobiotic, cardio protective and vasodilatory effects [3].

Plant phytochemicals have been associated with health benefits. Phenolic compounds in plant can generally be divided into three groups: phenolic acids, flavonoids and tannins. One of dietary antioxidant sources that are often used is purple sweet potato (PSP). Purple sweet potato (*Ipomoea batatas* L.) contains high amount of polyphenol and flavonoids such as anthocyanins. Anthocyanins are chemical substances with strong antioxidant properties that exhibit many

health-promoting effects such as anti-inflammatory, anti-diabetes, anti-aging and anti-hypertension [10] [11] and [12]. The antioxidant effect of PSP is approximately 2.5 times higher than those in berries. Phenolic compounds are mostly distributed between the potato cortex and skin (peel) tissues [13]. About 50% of the phenolic compounds are located in the potato peel and adjoining tissues, while the rest decrease in concentration from the outside toward the center of potato tubers. Antioxidant activity of a dairy food is important both for the shelf life of the product as well as for protection from oxidative damage in the human body [14]. Furthermore, milk contains a variety of antioxidant molecule traces *i.e.* low molecular weight thiols [15], ascorbate, tocopherol, retinol and carotenoids, tocopherol, retinol and carotenoids. The objective of this study was to evaluate the role of sweet potato peel (*Ipomoea batatas* L.) as hypocholesterolemic agent that can improve liver and kidney functions in rats.

2. Materials and Methods

2.1. Chemicals

Ammonium persulphate, 1,1 diphenyl-2-picrylhydrazyl radical (DPPH), rutin and Gallic acid were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Folin-Ciocalteus phenol reagents were purchased from Fluka Chemical Co. All other chemicals used were analytical grade.

2.2. Preparation of Sweet Potato Peel Powder and Peel Extract

Sweet potato fruits were washed by water and peeled carefully by Carborandum. Whole peels were air dried in a ventilated oven at 40˚C for 48 h and ground to a fine powder.

Sweet potato peel extract (SPPE) was prepared according to Shiban [15] as follow: 10 g of sweet potato peels powder were separately blended for 2 min with 600 ml of 80% ethanol. The mixture was then left, in the dark; at room temperatures for one hour prior to filtration (Whatman No. 1) and centrifuged at 3500 rpm for 10 min. Extracts were kept at −20˚C until analysis.

2.3. Starter Cultures and Fermented Milk Manufacture

Starter cultures were obtained from Christian Hansen (Copenhagen, Denmark). The starter ATB was a standard yoghurt culture containing a mixed strain culture of *S. thermophilus* TH4, *L. acidophilus* La5 and *L. bulgaricus*. Yoghurt was prepared according to the methods of Anifantakis [16] and the SPPE was added at the ratios of 1%, and 2%, then 3% starter culture was added and the mixtures were incubated at 45˚C until the gel structure was formed. The gel was stirred after and stored at refrigerator (5˚C ± 1˚C) until used.

2.4. Estimation of Bioactive Components in SPPE

2.4.1. Determination of Total Phenolic Compounds (TPC)

Total phenolic compounds in the SPPE were measured by Jenway-UV-VIS

Spectrophotometer, based on a colorimetric oxidation/reduction reaction [17] using Folin-Ciocalteu reagent.

2.4.2. Determination of Total Flavonoids Content (TFC)

The concentration of flavonoids in SPP extracts were measured by using rutin as a reference compound [18].

2.4.3. Radical Scavenging Activity (RSA) of Extracts

The electron donation ability of the obtained extract was measured by bleaching of the purple colored solution of DPPH [19].

2.5. Identification of Phenolic Compounds by HPLC

The phenolic acids and flavonoid compounds of the SPPE were identified according to the method of Mattila *et al.*, [20].

2.6. Physical and Chemical Properties of Fermented Milk

Fermented cow's milk was chemically analyzed for moisture, total nitrogen, protein and fat as described by AOAC [21]. Viscosity of fermented milk samples was determined using Rotational Viscometer Type Lab. Line Model 5437. Results were expressed as Cant Poise (cP).

2.7. Organoleptic Properties

Ten trained panelists from the staff members of Food Science and Human nutrition Department, College of Agriculture and Veterinary Medicine, Qassim University, KSA used a quality-rating scorecard for the evaluation of flavour (50 points) body and texture (40 points) and appearance (10 points).

2.8. Experimental Design

Thirty white male albino rats were purchased from faculty of pharmacy, King Saud University (Kingdom of Saudi Arabia). All animals were kept under normal healthy conditions and fed on a basal diet for one week, and then the animals were allocated into five groups (each group of 6 rats) of approximately equal average body weight (120 - 150 g). In the first week, all groups were fed a basal diet composed of Casein (20%), sucrose (23%), Corn Starch (15%), powdered cellulose (5%), soybean (24%), corn oil (5%), mineral mix (6%), vitamin mix (1%), DL-Methionine (0.7%), Choline bitartrate (0.3%) according to AIN-93 guidelines [22]. The experimental animals were inspected and approved by the Animal Care and Use Committee of Qassim University before and during the experimental period.

After the adaptation period (7 days), one group continued feeding on the basal diet and served as negative control (C^-). Other groups were fed on high cholesterol diet (67 g basal diet + 31.75 g animal fat, 1% cholesterol and 0.25% bile's acid) for two weeks and were classified into sub groups as follow: Group (2) was fed on high cholesterol diet and served as a positive control (C^+). The third

group was fed on 10% fermented milk without any additives (K_1). The fourth group was fed on 10% fermented milk fortified with 1% SPPE (K_2) and the fifth group was fed on 10% fermented milk fortified with 2% SPPE (K_3). At the end of the experimental period (6 weeks), rats were fasted overnight anesthetized by diethyl ether, bled and sacrificed. Blood samples were collected and centrifuged at 3000 rpm/10min. Serum was collected and stored at −20˚C until analyzed. After decapitation, livers were rapidly dissected out; two liver samples were taken from all groups and labeled. One sample of liver tissues was stored at (−20˚C), until used for assays of triglycerides, activity and the other portion was kept for histopathology.

2.9. Biochemical Analysis of Lipids

Total Cholesterol, HDL and triglyceride levels were estimated in serum. LDL was calculated [23]. Liver enzymes, alanine amino transferase (ALT), aspartate amino transferase (AST) and total protein were determined [24] Kidney function was determined in serum creatinine and urea [25].

2.10. Histopathological Examination

Autopsy samples were taken from the livers of rats in different groups and fixed in 10% formol saline for 24 hours. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and then eosin stain for routine examination through the light electric microscope [26].

2.11. Statistical Analysis

All data were statistically analyzed using the general linear models procedure of the statistical analysis system SAS [27]. Significances of differences were defined at $p < 0.05$. All experiments as well as related analysis results were repeated three times and all obtained data are expressed as an average.

3. Results

3.1. Bioactive Components and Total Antioxidant Capacity of SPPE

Concentration of phenolic compounds, as can be predicted from **Figure 1**, when expressed as mg equivalent in chlorogenic acid/100 g (DW), varies from 294.82 to 314.38. The total flavonoid contents in the sweet potato peel ranged from 48.12 to 52.40 mg/g DW. The radical scavenging activity of the SPPE was found to be 92.46%.

3.2. Identification of Phenolic Compounds by HPLC

The concentration of each phenolic compound in SPPE is shown in **Figure 2**. There was great variation among the identified components. Phenolic acids identified in SPPE ranged from 6.40 to 112.24 mg/100g DW. Some isomers were distinguished by comparing their retention time with standard antioxidant compounds that have been previously studied under similar conditions. By

means of this HPLC method, several phenolic compounds were detected which included; vanillic acid, protocatechuic, coumaric acid, caffien, catechin, eryptochlorogenic acid three isomers of the dicaffeoylquinic acids.

In the present study and as shown in Figure 1 Phenolic compounds identified in sweet potato peels extract included; vanillic acid, protocatechuic, coumaric acid, caffien, catechin, cryptochlorogenic acid and three isomers of the dicaffeoylquinic acids.

3.3. Physical and Chemical Properties of Fermented Milk

The chemical composition of prepared fermented milk fortified with sweet potato

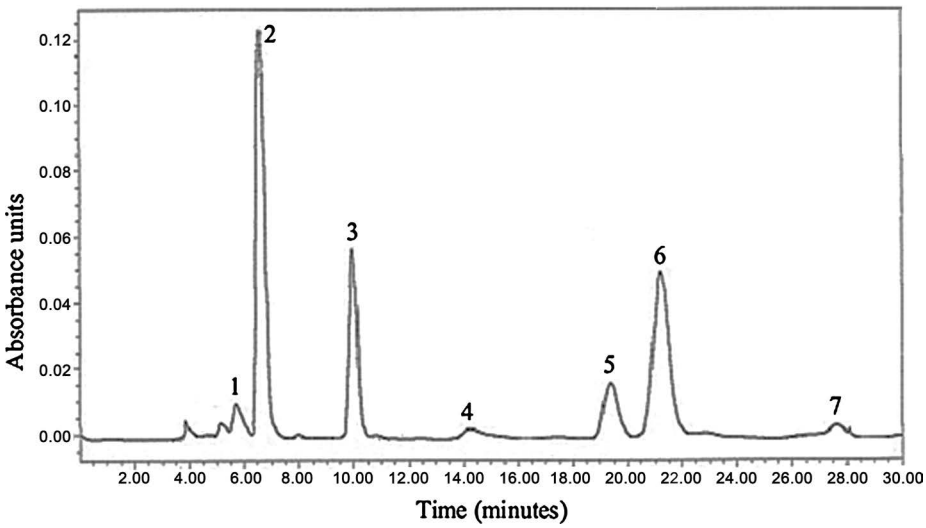

Figure 1. A representative chromatogram for a 20 ml sweet potato peel extract analyzed with a Nucleosil C_{18} 5 µm, 4 × 250 mm column using a mobile phase consisting acetonitrile: 10 mM phosphate buffer (80:20), pH 2.6, flow rate: 1 ml/min, temperature: 30˚C, UV detection at 220 nm.

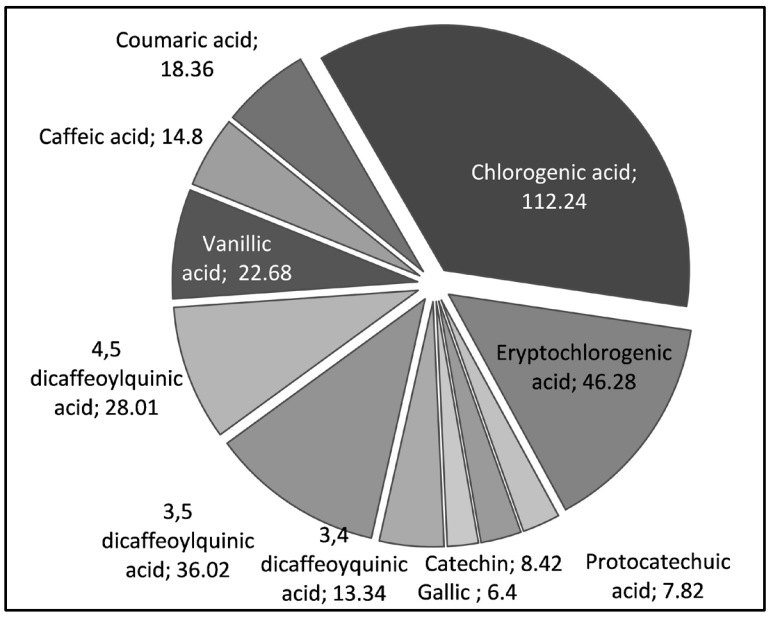

Figure 2. Phenolic acids in ethanol extracts of sweet potato peels as determined by HPLC.

peel is illustrated in Table 1. Fermented milk supplemented with SPPE was found to be characterized by a lower concentration of total solids (TS) and fat compared with control. Table 1 show that, Fermented milk without additives contained slightly higher fat contents when compared with other treatments. On the other hand, addition of SPP to milk did not affect the fat content of the resultant fermented milk. Furthermore, data showed that the fermented milk fortified with SPP resulted in slight increase in total protein (K_2 and K_3) compared with control. Similar finding were reported by Yousef *et al.*, [28] and Altemimi, [29].

Viscosity of fermented milk containing potato peel extract is shown in Table 1. Fermented milk containing SPPE was found to be significantly less viscous compared to the control sample.

3.4. Organoleptic Properties

Fermented milk fortified with different ratios of SPPE was subjected to sensory evaluation, and scores were recorded. As shown in Table 2 there was insignificant difference in flavor scores, and body & texture between treatments 1% and 2% SPPE when compared to other treatments. There were insignificant differences in appearance in all treatments. Blending improved fermented milk properties and over all acceptability. These results agree with the findings reported by Altemimi, [29] and Halim [30].

3.5. Improvement of Lipid Profile and Antioxidants of Hypocholesterolemic Rats by SSP

The effect of feeding of ethanol extract of SPP to hypocholesterolemic rats was

Table 1. Chemical analysis of fermented milk as affected by addition of sweet potato peels extract.

Treatments	Protein (%)	Fat/DM	Moisture (%)	Total nitrogen (%)	Viscosity (cPS)
C	3.48 ± 0.46^{b}	3.94 ± 0.24^{a}	87.76 ± 0.30^{b}	0.55 ± 0.040^{b}	5700 ± 14^{a}
K2	3.62 ± 0.42^{ab}	3.75 ± 0.28^{ab}	87.48 ± 0.32^{ab}	0.57 ± 0.041^{ab}	5260 ± 18^{b}
k3	3.84 ± 0.43^{a}	3.70 ± 0.26^{b}	87.02 ± 0.35^{a}	0.60 ± 0.040^{a}	5180 ± 13^{c}
LSD	0.319	0.0210	0.2739	0.211	3.408

Values with different letters in the same column are significantly different ($P \leq 0.05$); C: fermented milk without additives K_2: fermented milk fortified with 1% SPPE. K_3: fermented milk fortified with 2%. SPPE.

Table 2. Organoleptic properties of fermented milk as affected by fortified sweet potato peels extract.

Samples	Appearance (10)	Flavour (50)	Body and texture (40)	Total (100)
C	8^{a}	46^{b}	35^{a}	91^{c}
K2	9^{a}	48^{ab}	37^{ab}	94^{b}
K3	9^{a}	49^{a}	38^{ab}	96^{a}
LSD	0.334	0.0210	0.0458	0.3388

Values with different letters in the same column are significantly different ($P \leq 0.05$). K_2: fermented milk fortified with 1% SPPE. K_3: fermented milk fortified with 2%.SPPE.

shown in Table 3. When Fermented milk fortified with SPPE was administered to hypocholesterolemic rats for six weeks it prevented significantly ($p \leq 0.05$) the expected rise of serum TC, and LDL concentrations and resulted in a decrease in the level of TG when compared to rats of group (C^+).Also supplementation for six weeks increased significantly HDL concentration when compared to hypocholesterolemic rats fed on basal diet (G^+).

3.6. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Activities

The activities of serum AST and ALT are presented in Table 4. Oral feeding of rats with fermented milk containing SPPE at dose of 1% or 2% lowered significantly ($p < 0.05$) the elevation of serum enzymes compared to hypercholesterolemia control group (Table 4). In addition, serum concentration of total protein increased ($p \leq 0.05$) significantly after 6 weeks of feeding rats SPPE 1% and 2% respectively.

3.7. Renal Function in Hypocholesterolemic Rats Fed Fermented Milk Fortified with SPPE

Feeding rats with 10%, fermented milk containing SPPE prevented the rise of

Table 3. Effect of fermented milk fortified with Sweet potato peels extract on blood lipid profile of hypocholesterolemic rats (mg/dl).

Group	Triglycerides	T. Cholesterol	HDL	LDL
C^-	149.92 ± 3.41^b	126.24 ± 3.34^b	60.77 ± 1.01^a	35.48 ± 1.64^c
C^+	175.78 ± 1.65^a	157.64 ± 0.83^a	41.73 ± 014^c	80.76 ± 0.26^a
K_1	160.13 ± 3.69^a	129.89 ± 0.16^b	50.81 ± 0.53^b	47.06 ± 1.10^b
K_2	150.22 ± 0.67^b	110.59 ± 0.97^c	60.74 ± 4.29^a	19.81 ± 0.07^d
K_3	137.00 ± 1.47^b	102.46 ± 1.68^b	61.09 ± 0.86^a	13.97 ± 0.53^d
LSD	9.8684	5.2315	3.7065	4.3837

Values with different letters in the same column are significantly different ($P \leq 0.05$); C^-: Negative control group, fed on basal diet period; C^+: Positive control group hypocholesterolemic rats and fed on basal diet; K_1: Hypocholesterolemic rats fed on 10% fermented milk without any additives; K_2: Hypocholesterolemic rats fed on 10% fermented milk fortified with 1% SPPE; K_3: Hypocholesterolemic rats fed on 10% fermented milk fortified with 2% SPPE.

Table 4. Effect of fermented milk fortified with Sweet potato peels extract administration on liver and kidney functions of hypercholesterolemic rats.

Groups	T. protein	ALT(U/L)	AST(U/L)	Creatinine (mg/dl)	Urea(mg/dl)
C^-	6.82 ± 0.11^a	67.50 ± 0.70^a	57.50 ± 0.70^b	0.755 ± 0.02^b	43.06 ± 79^c
C^+	5.69 ± 0.28^b	77.50 ± 2.12^a	87.50 ± 2.11^a	1.18 ± 0.05^{ab}	45.58 ± 0.62^b
K_1	6.97 ± 0.79^a	72.00 ± 1.41^a	88.00 ± 1.41^a	1.48 ± 0.68^a	42.39 ± 0.55^a
K_2	6.95 ± 0.77^a	57.50 ± 1.41^b	75.00 ± 1.41^b	1.22 ± 0.02^{ab}	41.42 ± 0.11^a
K_3	6.73 ± 0.86^a	57.00 ± 0.72^b	74.00 ± 2.82^b	1.14 ± 0.02^{ab}	40.58 ± 0.68^a
LSD	1.3613	1.9979	1.9979	0.6286	0.7398

Values with different letters in the same column are significantly different ($P \leq 0.05$).

mean serum creatinine and urea concentrations. The rate of prevention was proportional to the concentration of SPPE (Table 4).

3.8. Effect of Feeding Fermented Milk on Liver Histopathological Alterations of Experimental Rats

The microscopic examination of liver, tissues in hypocholesterolemic rats and the consequences of administration of SPPE in other treated groups are shown in Figure 3.

As presented in Figure 3 in liver of positive control group (b) the hepatic parenchyma showed acute cell swelling and few of them still revealed apoptotic changes. The hepatic blood vessels and sinusoid were dilated and congested. Portal and interstitial lymphocytic aggregations could be seen. A few portal areas showed intense fibrosis, edema, congestion and proliferative bile duct tubes beside hyperplastic kuffer cells. Liver of Hypocholesterolemic rats fed on 10% fermented milk without any additives (c) showed portal and interstitial lymphocytic aggregations and infiltrations and portal fibroses. While liver of Hypocholesterolemic rats fed on 10% fermented milk fortified with 1% SPPE (d) showed interlobular fibrosis with hylanized hepatic arteriole and cell swelling of hepatic cells were seen. In Hypocholesterolemic rats fed on 10% fermented milk fortified with 2% SPPE (d) the majority of the hepatic parenchyma revealed normal histological structures. Single portal area has slightly moderate bile duct proliferation

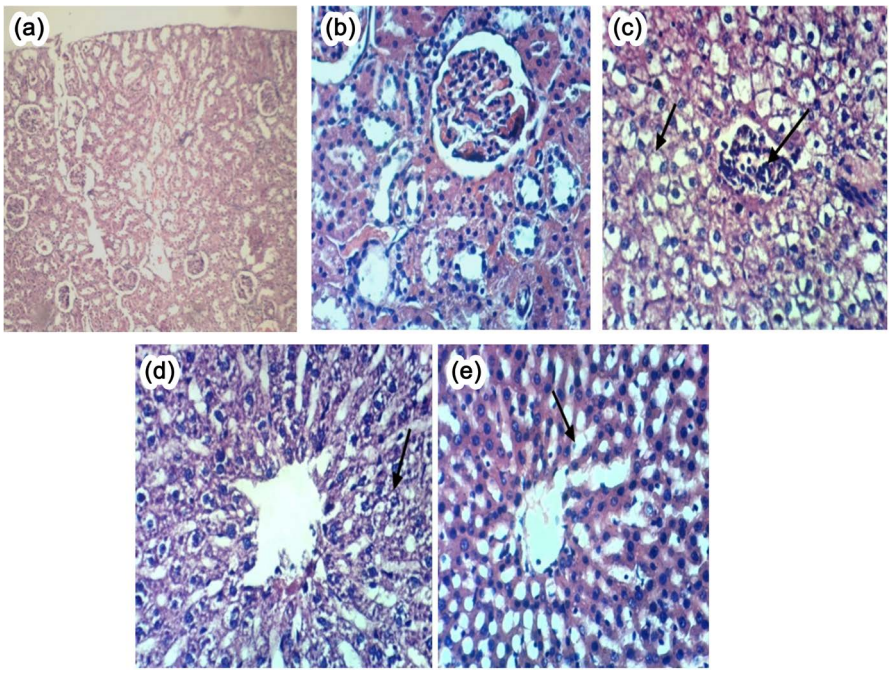

Figure 3. Microscopic examination of liver tissue using (H & E ×300): (a) Liver tissue of normal (negative control) of rat (×300); (b) Liver tissue of hypocholesterolemic rats (positive control of group); (c) Liver tissue of hypocholesterolemic rat and fed on 10% fermented milk without any additives (k_1); (d) Liver tissue of hypocholesterolemic rat and fed on 10% fermented milk fortified with 1% SPP (k_2) and (e) Liver tissue of hypocholesterolemic rat and fed on 10% fermented milk fortified with 2% SPP (k_3).

beside congestion of hepatic blood vessels in liver. These may be due to a high concentration of cholesterol being able to cause degeneration of hepatocytes.

4. Discussion

Results revealed that SPPE have a high antioxidant potential and inhibitory action against lipid oxidation SPPE is rich in phenolic compounds however, great genetic variability was reported for sweet potatoes [31] [32], and the highest concentrations are usually observed in purple sweet potato. Therefore, sweet potato peel is a good source of bioactive compounds, with high antioxidant properties. Flavonoids and tannins that were found in the plant are phenolic compounds that act as primary antioxidants or free radical scavengers [33]. Ethanol extract of SPP showed good antioxidant activities. In the present study, SPPE showed a significant variability in their inhibitory activity against DPPH radical that ranged from 22.29 to 28.57 μmole TE/100 g DW. However, regarding the antioxidant capacity, a wide variation was reported among the sweet potato geno-types, it was noticed that purple are considered the best in this respect. [32]

It was reported that Seven types of phenolic acids have been found in sweet potato including: neochlorogenic acid, cryptochlorogenic acid, chlorogenic acid, gallic acid and three isomers-isochlorogenic acid A (3,5-diCQA), isochlorogenic acid B (3,4-diCQA) and isochlorogenic acid C (4,5-diCQA). Results of the present study are in line with the results of Arun [13].

Examination of Fermented milk containing SPPE was found to be significantly less viscous compared to the control sample. The decrease in viscosity was proportional to the level of additives. This may be due to the fact that addition of plant extracts generally decreases the viscosity of the products owning to reduced water-binding capacity of proteins. No significant differences in sensory evaluation scores were noticed in fermented milk fortified with different ratios of SPPE. As shown in **Table 2** there was insignificant difference in flavor scores, and body & texture between treatments 1% and 2% SPPE when compared to other treatments. There were insignificant differences in appearance in all treatments. Blending improved fermented milk properties and over all acceptability. These results agree with that reported by Halim [30].

The decrease in the levels of total cholesterol, triglycerides and LDL, as compared to the control, may be due to the fact that SPPE is rich in antioxidants particularly phenolic compounds that can prevent and lower the free radicals level and lower liver LDL-cholesterol uptake and protect the cortical neurons from cell death. Similar findings were reported [11] [12] [10] and [13]. These are very important findings because Lowering of serum lipid concentration through dietary or drug therapy seems to be associated with a decrease in the risk of vascular diseases [32]. However, dietary therapy seems to be safer and cheaper when compared with drug therapy.

The initial decreased levels of total proteins may be due to the liver cell injuries induced by high fat diet. Blending with SPPE significantly increased the

concentration of serum total protein and lowered the activities of ALT, and AST levels. SPPE is rich in antioxidants that can attenuate and lower the free radicals levels. [31] Reported that the beneficial effects of these phytochemicals in preventing the ethanol-induced hepatotoxicity are mediated by the antioxidant effects.

Milk containing SPPE showed antioxidant properties [32]. Dietary potato peel intake and potato peel components have been suggested as protection and prevention of chronic diseases, particularly for protection against cardiovascular diseases and certain cancers [33]. Emerging evidence supports additive and/or synergistic effects of potato peel components for protection against certain cancers. The obtained results are in agreement to those reported before Hassan [34] and Ghalehkandi [35].

Feeding high fat diet resulted in histopathological changes in the liver tissues and that may be due to liberation of lipid peroxides that may cause damage to the cell membrane and making the membrane fragile [36] and [37]. However, feeding in fermented milk containing SPPE showed sign of recovery liver cells from acute cell swelling, edema, dilation and congestion of blood vessels and sinusoids.

5. Conclusion

It can be concluded that SPPE is rich in antioxidants and can be recommended as functional food to treat hypercholesterolemia, reduce the risk of atherosclerosis and improve liver functions. In the future we are going to study the beneficial effect of adding SPPE as natural antioxidants to fatty dairy products and fermented foods.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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