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ORIGINAL ARTICLE

Effects of repeatedly heated cooking oil consumption in mice: a study on health implications

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Abstract

Background: Cooking oils are a major part of human diets, but repeated use of heated oils can have detrimental effects on consumer health. This study aims to investigate the impact of different heating grades of vegetable oils on the hemato-biochemical parameters and vital organs like the heart, liver, kidney, and intestine in mice.

Methods: Thirty mice were randomly assigned to different treatment groups, including a control group (diet only), unheated cooking oil (UHCO) group, single heated cooking oil (SHCO) group, three times repeatedly heated cooking oil (3RHCO) group, and repeatedly heated cooking oil (ReHCO) group. Blood and organ samples were collected on day 31 to investigate hemato-biochemical parameters and histo-morphological alterations in response to the oil treatments.

Results: The oil-treated groups showed significant ($P<0.05$) decreases in the total erythrocyte, leukocyte, and hemoglobin levels. Meanwhile, serum levels of total cholesterol, triglyceride, high-density lipoprotein, glucose, and creatinine increased significantly ($P<0.05$), while low-density lipoprotein and protein levels dropped markedly in the treatment groups. Severe histo-morphological alterations were also found in the liver (hepatocytic degeneration with hydropic change in the 3RHCO and ReHCO groups), kidney (glomerular atrophy with increased glomerular space, tubular degeneration, and lymphocytic infiltration in the SHCO, 3RHCO, and ReHCO groups), and colon (lymphocytic infiltration in the mucosal layer of ReHCO group).

Conclusions: These findings suggest that the consumption of heated oils can have severe adverse effects on consumers' health, leading to alterations in blood chemistry and damage to vital organs.

Key Words: Cooking oil, Repeated heating, Mice, Hemato-biochemical profile, Vital organs.

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Introduction

In our fast-paced society, eating habits have shifted towards consuming meals outdoors, often preferring deep-fried foods over homemade alternatives (Vaskova and Buckova, 2015). The widespread availability and affordability of deep-fried dishes have made them immensely popular worldwide due to their delicious flavors. Vegetable cooking oils, being the primary source of lipids and a crucial component of biological membranes, play a vital role in the human diet (Vaskova and Buckova, 2015). These oils are commonly used to improve the palatability of cooked dishes (Warner, 2004). However, high temperatures during cooking can lead to oil oxidation (Oboh *et al*., 2014). Edible vegetable oil is a key component of fried foods, and its cost-effectiveness often leads to its repeated use. Unfortunately, high-heat cooking generates hydroperoxides and aldehydes, which can infiltrate the food and subsequently enter the gastrointestinal tract and systemic circulation (Ambreen *et al*., 2020). Regular consumption of repeatedly heated frying oils has been linked to an increased risk of developing hypertension (Ng *et al*., 2012). Reusing frying oil poses significant health hazards, including histopathological changes and genetic material modifications (Venkata and Subramanyam, 2016). During cooking, free radicals generated by lipid peroxidation can damage membrane lipids, potentially leading to oxidative stress-induced alterations in genetic materials (Siddiq *et al*., 2019).

Palm oil, being cost-effective, is commonly used in Bangladesh's hotels and restaurants for frying and meal preparation (De Marco *et al*., 2007). Repeated heating at high temperatures results in various physical changes, including increased density, altered color, and the development of rancid odor (Ambreen *et al*., 2020). High-heat treatments induce oxidation, hydrolysis, and polymerization processes in oils (Choe and Min, 2007) leading to the formation of hazardous elements like peroxides (Leong *et al*., 2012) and trans-fat (Kemény *et al*., 2001). Previous experiments involving male ICR mice fed a standard diet supplemented with 25% used oil for 12 weeks resulted in hepatic cell swelling and necrosis (Chang *et al*., 2021). Oxidative stress caused by free radicals can impact numerous inflammatory conditions, leading to various glomerular lesions triggered by mediators such as cytokines and chemokines. These mediators activate leukocytes, generate reactive oxygen species, and exacerbate glomerular damage (Hashem and Salama, 2012).

Palm, soybean, mustard, sunflower, and rice bran are commonly consumed edible oils globally (Zhou *et al*., 2020). Fresh oil generally has minimal or no adverse effects on blood pressure or cardiac health. However, regular consumption of repeatedly heated palm oil has been associated with elevated blood pressure and cardiac tissue necrosis (Leong *et al*., 2012). Diets high in thermally oxidized palm oil have been linked to anemia due to decreased hemoglobin levels (Mesembe *et al*., 2004). Palm oil can lower total cholesterol, LDL cholesterol, and triglycerides in the bloodstream (Edem, 2002). Additionally, heated palm oil can temporarily alter lipid profiles and increase serum LDL-cholesterol levels.

Currently, different animal models, such as rats and mice, are used to investigate pathological conditions induced by dietary fat consumption. The choice of experimental animal models is crucial when investigating the impact of repeatedly heated cooking oils on lipid metabolism and oxidative stress. In this context, to investigate the impacts of repeatedly heated edible oil on human health, the mouse model stands out as a valuable tool due to its unique biological features, particularly its similarity to humans in terms of lipid metabolism. Given the considerations mentioned above, this study aims to investigate the impacts of different grades of heated oil on hemato-biochemical indices and histo-morphologic attributes in the heart, liver, kidney, and colon of mice.

Materials and Methods

Ethics statement

The current study was solely conducted following the institutional guidelines for using the animal model for research purposes. The experimental procedure was authorized by the Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University (BAU), Bangladesh. The authorization number is - AWEEC/BAU/2023(50).

Animal model

Thirty apparently healthy Swiss albino mice were obtained from icddr,b in Dhaka, Bangladesh. These mice were in good health and free from external deformities.

Experimental design

We used 30 six-week-old Swiss albino mice, divided into five groups through random selection, with six replications per group. The mice were then assigned to different treatments: untreated control mice (diet only), mice treated with unheated cooking oil (UHCO), single heated cooking oil (SHCO), three times repeatedly heated cooking oil (3RHCO), and repeatedly heated cooking oils collected from restaurants (ReHCO). The different oils were administered orally via gavage for 31 days at a rate of 0.2 ml per mouse.

Preparation of oil sample for treatment

We used one liter of freshly purchased cooking oil as UHCO. Another four liters of oil were heated above their smoke point (above 300°C) for 30 min and then cooled to room temperature. One liter of this heated oil was separated and labeled as SHCO. We repeated this process to obtain three times repeatedly heated cooking oil (3RHCO). Additionally, one liter of restaurant-heated oil (ReHCO), which had been heated and used multiple times, was collected from a restaurant.

Hemato-biochemical analysis

We collected 3 ml blood samples via cardiac puncture from mice that had fasted overnight. Hemoglobin (Hb) concentration (gm %) was determined using the cyanomethemoglobin technique (Drabkin and Austin, 1932). Total erythrocyte counts (TEC, million/mm3) were measured using a modified version of Natt and Herrick's method (Natt and Herrick, 1952). For serum lipid profile analysis, including total cholesterol (TC, mg/dl), triglycerides (TG, mg/dl), high-density lipoprotein (HDL, mg/dl), low-density lipoprotein (LDL, mg/dl), protein profile (g/dl), creatinine (mg/dl), and serum glucose, we used a spectrophotometric method with a Humalyzer 2000 (Human type, Germany).

Histopathological investigation

On day 31, the mice were sacrificed, and the liver, heart, kidney, and intestine (colon) samples were collected. These tissue samples were processed and stained with Hematoxylin and Eosin (H&E) stain for histopathological examination.

Results

Hematological indices

The control group showed the highest levels of TEC, TLC, and Hb, while the ReHCO group had the lowest. There was a reverse linear relationship between the heating frequency and TEC, TLC, Hb levels. SHCO, 3RCHO, and ReHCO groups had significantly lower TEC, TLC, and Hb, with TLC also dropping markedly in the UHCO group compared to the control group (Figure 1).

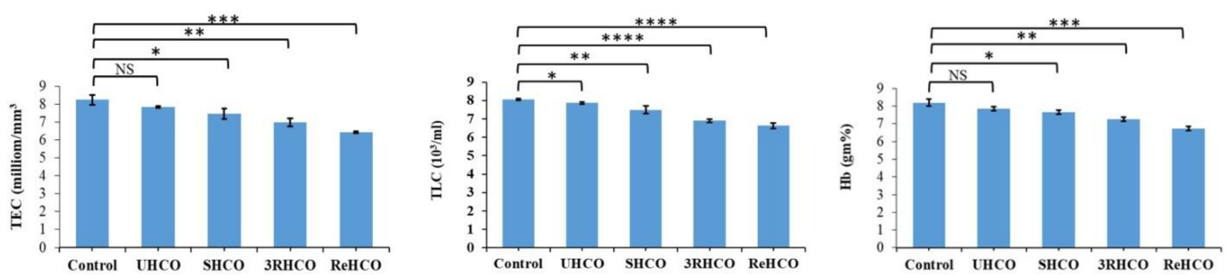

Figure 1. Total erythrocyte count (TEC), total leukocyte count (TLC), and hemoglobin (Hb) concentration in different groups of mice. Data are presented as mean ± SD. ****= Statistically significant at 0.01% level (P < 0.0001), ***= Statistically significant at 0.1% level (P < 0.001), **= Statistically significant at 1% level (P < 0.01), *= Statistically significant at 5% level (P < 0.05), and NS= non-significant.

Sero-biochemical indices

The serum lipid profile is presented in Figure 2 a. TC, TG, and HDL levels were significantly higher ($P < 0.05$) in all treatment groups compared to the control. Although TC levels increased with oil heating frequency, no significant variation ($P > 0.05$) was observed between treatment groups. The ReHCO group had the highest TC, TG, and HDL levels, followed by 3RHCO, SHCO, UHCO, and the control group. On the contrary, serum LDL levels significantly ($P < 0.05$) dropped in the treatment groups in comparison to the control with the ReHCO group having the lowest serum LDL. Serum glucose and creatinine levels significantly increased in all oil-treated groups, while total protein and albumin levels markedly dropped ($P < 0.05$) in the treatment groups (Figure 2 b).

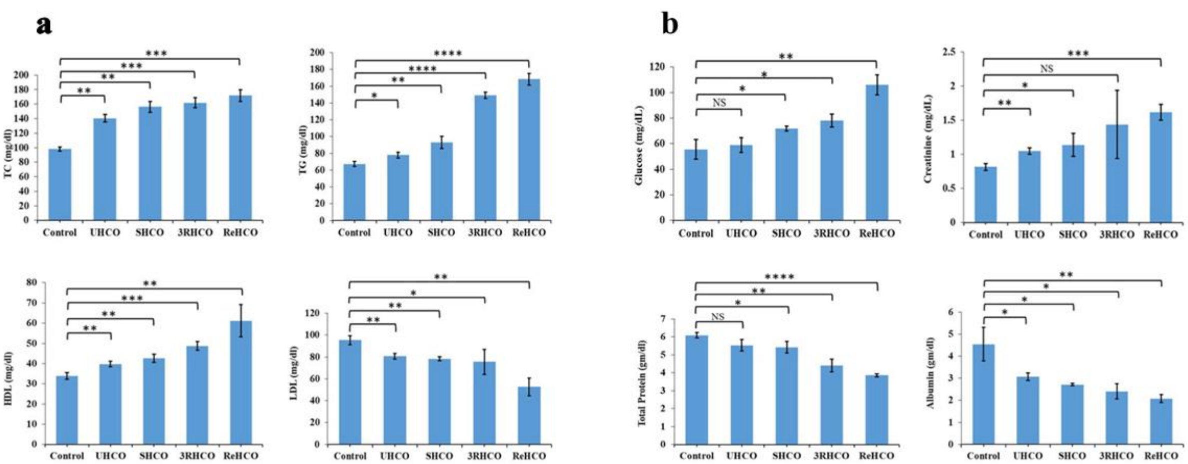

Figure 2 (a). Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) in different groups of mice. Data are presented as mean ± SD. ****= Statistically significant at 0.01% level ($P < 0.0001$), ***= Statistically significant at 0.1% level ($P < 0.001$), **= Statistically significant at 1% level ($P < 0.01$), *= Statistically significant at 5% level ($P < 0.05$), and NS=non-significant. (b) Serum glucose, creatinine, total protein, and albumin levels in different groups of mice. Data are presented as mean ± SD. ****=Statistically significant at 0.01% level ($P < 0.0001$), ***= Statistically significant at 0.1% level ($P < 0.001$), **= Statistically significant at 1% level ($P < 0.01$), *= Statistically significant at 5% level ($P < 0.05$), and NS=non-significant.

Histopathological alterations

The histopathologic features of the heart, liver, kidney, and colon are shown in Figure 3. The heart in both control and treated groups showed normal histoarchitecture, with intact muscle fiber, intercalated disc, and nuclei. However, in the liver of treatment groups, distinct alterations were evident. The SHCO group showed localized hepatocyte degeneration near the central vein region, creating empty spaces. The 3RHCO group exhibited a loss of cellular integrity in hepatocytes, while the 3RHCO and ReHCO groups showed hepatocytic degeneration with hydropic changes Kidney alterations were observed in the 3RHCO and ReHCO groups, including glomerular atrophy with increased glomerular space, lymphocytic infiltration, glomerular, and tubular degeneration. UHCO and SHCO groups showed no noticeable changes. In the colon, no significant alterations were observed in the oil-treated groups except for the ReHCO group, which displayed nodular aggregation of inflammatory cell infiltration in the mucosa.

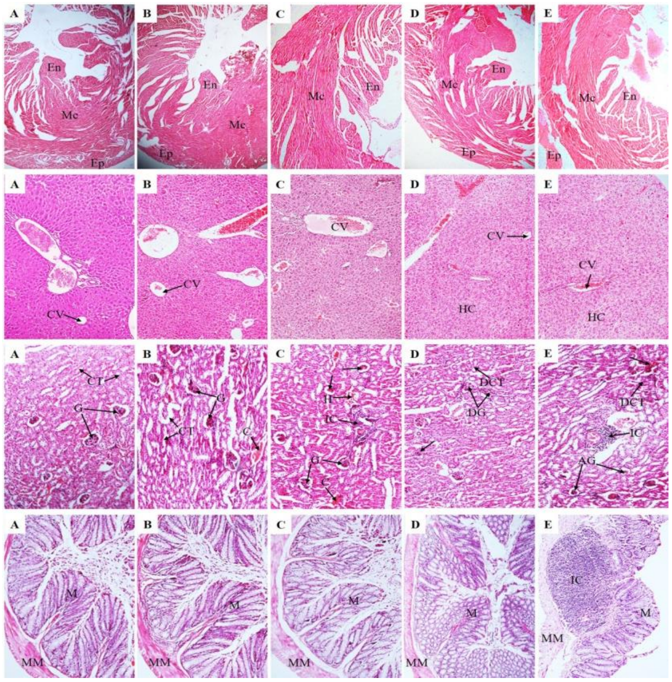

Figure 3. Histoarchitecture of heart, liver, and kidney of mice in different groups (H&E stain). (A) Control, (B) UHCO, (C) SHCO, (D) 3RHCO, and (E) ReHCO. AG - atrophied glomerulus, C - congestion, CT - convoluted tubules, CV - central vein, DCT - degenerated convoluted tubules, DG - degenerated glomerulus, En - endocardium, Mc - myocardium, Ep - epicardium, G - glomerulus, HC - hydropic change, IC - inflammatory cells, M - mucosa, MM - muscularis mucosa.

Discussion

Vegetable oil is a key nutritional ingredient in our daily meals. However, frequent heating can lead to lipid oxidation, potentially diminishing its health benefits. Concerns are growing regarding the cardiovascular and potential carcinogenic effects of edible oils due to this oxidation. The hematological analysis showed significant reductions in TEC, TLC, and Hb concentration in the oil-treated mice which are consistent with previous reports (Mesembe *et al.*, 2004; Venkata and Subramanyam, 2016). Interestingly, an earlier study involving heated edible oils found no impact on blood cellular proportions (Alexander, 1981). Mice exposed to different oil treatments showed marked increases in TC, TG, and HDL levels, aligning with the earlier studies (Mesembe *et al.*, 2004; Morshed *et al.*, 2018; Siddiq *et al.*, 2019). This increase in lipid parameters, especially in a dose-dependent manner, raises significant health concern. Contrary to some studies suggesting a decrease in TC levels with heated oil consumption (Hur *et al.*, 2005), our findings showed an upward trend in serum TC levels in the oil-treated groups. Serum LDL levels significantly dropped in the treatment groups, contradicting reports of increased LDL levels following oil treatment (Jaarin *et al.*, 2006; Siddiq *et al.*, 2019). Elevated serum lipid content is a risk factor for cardiovascular diseases. Elevated serum TC and TG levels have been associated with an increased risk of myocardial infarction, which can ultimately lead to cardiovascular diseases (Langston *et al.*, 2011). Interestingly, a high level of HDL, traditionally considered protective against cardiovascular diseases, may have adverse effects on cardiac health when present in excessive quantities, as suggested in a recent study report (Franczyk *et al.*, 2021). Moreover, high levels of serum LDL have been linked to a reduced rate of survival due to their accumulation in cardiac and arterial walls, potentially leading to severe hypertension (Kalantar-Zadeh *et al*., 2002). Serum glucose and creatinine levels were increased in the heated oil-treated groups that is in line with earlier reports (Venkata and Subramanyam, 2016; Morshed *et al*., 2018). These increases are a significant health concern (Venkata and Subramanyam, 2016). On the contrary, serum protein levels markedly dropped in the oil-treated groups, although there are no previous reports of such a decrease in serum protein levels.

In the histomorphological investigation, no significant alterations were observed in the hearts of the oil-fed mice, contrasting earlier studies that reported congested hearts, myocardial degeneration, and vacuolation in the cardiac muscle (Shastry, 2011; Morshed *et al*., 2018). In the liver, the heated oil treatment groups showed hepatocyte degeneration, loss of cellular integrity, and hydropic changes, consistent with previous reports (Shastry, 2011; Morshed *et al*., 2018). Particularly, the vacuolation of hepatocytes in response to heated oil consumption in a dose-dependent manner has been reported (Venkata and Subramanyam, 2016). The kidney histomorphology showed marked alterations, characterized by glomerular atrophy with increased glomerular space, tubular degeneration, and lymphocytic infiltration. Similar marked organ alterations in response to oil treatments have been reported previously (Shastry, 2011, Morshed *et al*., 2018).

Hemorrhage in the mucosal layer of the colon was observed in the present study. A similar study by (Venkata and Subramanyam (2016)) reported colon polyps in rats treated with single-heated cooking oil (SHCO) and three times repeatedly heated cooking oil (3RHCO). These colon polyps were mostly adenomas. Heating of edible cooking oils results in oxidative stress resulting from lipid peroxidation and the formation of free radicals (Ambreen *et al*., 2020). These free radicals interact with cell membrane lipids, causing damage to cell membranes and cellular integrity (Venkata and Subramanyam, 2016). These alterations in the cellular levels of the liver, kidney, and colon may contribute to the observed changes in the hemato-biochemical profile in mice.

Consumers are consistently exposed to various toxic substances through different food items. Hence, in developing countries like Bangladesh, the results of this research can provide valuable insights into the practical aspects of these health issues that have been previously overlooked. Additionally, these findings can contribute to a shift in the paradigm toward selecting study models that more accurately simulate human health in the context of developing countries.

Conclusions

This study specifically investigated the effects of consuming various levels of heated oils on hemato-biochemical indices and histo-morphological alterations in the heart, liver, kidney, and intestine of mice. Based on our findings, it is evident that the consumption of heated edible oils can significantly compromise the health status of consumers by altering blood chemistry and causing damage to vital organs. In conclusion, our study strongly discourages the consumption of repeatedly heated vegetable oils based on the results we have observed. However, we also recommend further research to analyze the oxidative status of mice in response to the consumption of heated oils.

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Conflict of interest

The authors have declared no conflict of interest.

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