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In *Vivo* Toxicity Assessment of The Effects of Heated Palm oil on Mice

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Abstract

Palm oil is a common cooking oil, but repeated heating can induce harmful chemical changes. This study investigated the adverse effects of fresh and heated palm oil on oxidative stress, liver histopathology, and genotoxicity *in vivo* using an mice model. Thirty male CD-1 albino mice (*Mus musculus*) were equally divided into three experimental groups: a control group receiving no treatment, a group treated with fresh palm oil and a group treated with heated palm oil for two weeks, five days a week. Body weight measurements showed distinct patterns among groups, with group treated with fresh palm oil demonstrating the highest weight gain (27.9%) compared to controls (25.2%), while group treated with heated palm oil displayed severely attenuated growth (1.74%). The micronucleus assay in bone marrow cells confirmed significant genotoxic potential of thermally oxidized Palm oil. Histopathological examination of liver tissues demonstrated well-preserved hepatic architecture in control group, whereas group treated with fresh palm oil exhibited mild steatosis and sinusoidal dilation. In contrast, group treated with heated palm oil presented severe hepatocellular damage characterized by extensive necrosis, inflammatory infiltrates, pronounced steatosis, and vascular congestion. Biochemical analysis revealed that group treated with heated palm oil exhibited significantly elevated malondialdehyde levels accompanied by markedly reduced activities of key antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase, indicating substantial oxidative stress induction. These findings collectively demonstrate that while fresh palm oil may promote adiposity with minimal hepatic effects, heated-palm oil induces profound oxidative damage, hepatotoxicity, and DNA damage, underscoring the critical public health implications of consuming repeatedly heated palm oils in dietary practices. Based on these results, we strongly recommend avoiding the use of repeatedly heated palm oil in cooking practices due to its potential health risks. Instead, we suggest exploring alternative, safer oils for culinary purposes to mitigate the adverse effects associated with heated palm oil consumption. By adopting healthier cooking oil options, individuals can reduce their exposure to harmful compounds and promote overall well-being.

Key Words

Palm oil, Bone marrow cells, Liver cells, Micronucleus Test, Oxidative Stress Markers

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1. Introduction

Palm oil (Po), a popular consumed edible vegetable oil, has been a staple in many cuisines for centuries. Derived from the fruit of the *Elaeis guineensis* tree, Po has been used for approximately 5,000 years. Its global production has increased significantly, with Malaysia producing 4.5 million tons of crude Po in 1988, accounting for 58% of global production (Karolina *et al.*, 2019, 90). Po is categorized into two types: palm stearin and palm olein, with palm olein comprising approximately 50% saturated fatty acids (SFA), 50% monounsaturated fatty acids (MUFA), and small amounts of polyunsaturated fatty acids (PUFA) (Cottrell, 1991, 989-1009).

Additionally, Po is rich in vitamins A and E, potent antioxidants that help prevent ischemic damage and protect against arterial plaque formation. When consumed as part of a well-balanced diet, Po supports heart health without increasing the cardiovascular disease risk (Odia *et al.*, 2015, 9-144). However, repeated heating of Po is a common practice driven by cost-saving measures (Azman *et al.*, 2012, 91-101). The oil's affordability and excellent frying properties caused a notable rise in its consumption in many countries over the past few decades (Sun *et al.*, 2015, 58-1549). Thermal processing, particularly repeated heating, can compromise the oil's nutritional quality by causing oxidative damage, which significantly reduces its carotenoid content and associated health benefits (Oboh *et al.*, 2014, 59-65). Furthermore, Hydroperoxides and aldehydes are among the harmful substances produced by high-temperature frying that can be absorbed by food and enter the bloodstream when consumed. (Grootveld *et al.*, 1998, 8-1210). This process also leads to lipid peroxidation and the formation of trans fatty acids, ultimately disrupting enzyme activity and antioxidant balance (Patsioura *et al.*, 2017, 84-99; Perumalla et al., 2016,637-643; Ju *et al.*, 2019, 37). Thermally oxidized oils contain complex compounds, including oxidized monomers, dimers, and polymers, which alter lipid properties (Serjouie *et al.*, 2010, 310-323). The widespread practice of reusing frying oil for economic reasons carries significant health risks. The consumption of heated Po has been linked to various health problems, including hypertension (Soriguer *et al.*, 2003, 22-1015)., endothelial dysfunction (Leong et al., 2010, 9-66; Williams et al., 1999, 5-1050), and histological abnormalities (Leong *et al.*, 2008, 72-567; Farag *et al.*, 2010, 501,509). Furthermore, studies have shown that heated Po can cause organ damage, including harm to the kidneys, liver, heart, and intestinal mucosa (Alaam *et al.*, 2012, 120-130; Boniface *et al.*, 2012, 759-767). Oxidized Po is often used instead of its raw form, but prolonged heating leads to lipid oxidation, which may be harmful and carcinogenic to tissues (Asare *et al.*, 2013, 51-1948). This study aimed to investigate the *in vivo* toxicity of heated Po and its potential negative effects on mice. Specifically, we assessed the effects of oral treatment with fresh and heated Po on liver by using histopathological analysis, oxidative stress markers analysis, and on bone marrow by using micronucleus test. This study offers insightful information about the possible health risks associated with consuming heated Po, ultimately contributing to a better understanding of its toxicological profile and informing strategies for safe consumption.

2. The Theoretical Framework

According to **Jayaraman *et al.*** (**2021**), palm oil (Po) is widely used in the manufacturing of food, cosmetics, and biofuels, making it one of the most widely used vegetable oils globally. **Chong *et al.*** (**2018**) explained that Po is extracted from the fruit of the *Elaeis guineensis* tree and is recognized for its high productivity and economic value in contrast to alternative vegetable oils. **Ambreen *et al.*** (**2020**) reported that hydroperoxides and aldehydes are produced during high-temperature cooking and can enter food matrices before being absorbed into the gastrointestinal tract and systemic circulation. **Kamsiah *et al.*** (**2016**) reported that repeatedly heating Po alters its chemical structure, leading to the formation of harmful compounds, including trans fats and free radicals. **Adam *et al.*** (**2008**) reported that free radicals produced during the frying process can initiate lipid peroxidation, leading to lipid degradation. Malondialdehyde (MDA), a key secondary oxidation byproduct of peroxidized polyunsaturated fatty acids (PUFAs), plays a significant biological role. **Choe *et al.*** (**2006**) demonstrated that when Po is heated, it undergoes hydrolysis, oxidation, and polymerization, leading to the

formation of reactive oxygen species (ROS) such as hydroperoxides and low-molecular-weight volatile compounds. These compounds contribute to raise oxidative stress. While the food is being fried ROS are absorbed into the food, potentially posing health risks upon consumption. **Ong *et al.* (2020)** demonstrated that excessive heating leads to the formation of acrylamide and polycyclic aromatic hydrocarbons (PAHs), both of which have been linked to cancer-causing impact. **Oguntibeju *et al.* (2009)** reported that the intake of fresh Po has been shown to induce beneficial physiological and biochemical effects that contribute to a reduced incidence of cardiovascular, kidney, liver, and lung diseases. Furthermore, research has demonstrated that fresh Po extract holds significant economic and biological value .**Narang *et al.* (2004)** reported that fresh Po is an abundant source of natural antioxidants, particularly tocotrienols, which have been shown to exert beneficial effects in mitigating oxidative stress associated with hypertension .**Medeiros *et al.* (2005)** also demonstrated that the prolonged consumption of fresh Po has a positive impact on lowering blood pressure in spontaneously hypertensive rates. **Bayorh *et al.* (2002)** revealed that fresh Po reduced oxidative stress-induced hypertension by elevating prostacyclin and nitric oxide levels and decreasing thromboxane A_2 and isoprostane levels .**Che Idris *et al.* (2020)** demonstrated that fresh Po exhibits hypocholesterolemic and anti-atherogenic properties, suggesting its potential benefits for human health.

Halliwell *et al.* (2015) revealed that long-term consumption of oxidized fats and oils has been linked to a number of harmful health outcomes, such as fatty liver disease, anemia, blood clot formation, impaired growth, essential fatty acid deficiency, and disruption of nucleic acids involved in important metabolic enzyme functions. **Leong *et al.* (2008)** reported that consumption of heated Po resulted in elevated blood pressure and cardiac tissue necrosis, whereas fresh Po did not exhibit these adverse effects . **Kamsiah *et al.* (2016)** concluded that prolonged intake of repeatedly heated Po contributes to elevated low-density lipoprotein (LDL) cholesterol while decreasing high-density lipoprotein (HDL) cholesterol levels, a key factor in atherosclerosis. **Boniface *et al.* (2014)** reported that palm oil has the possibility to cause organ failure and induce histopathological alterations in various organs, including the heart, intestinal mucosa, liver, and kidneys. **Choe *et al.* (2007)** found that the decline in renal function involves oxidative stress, in which an overproduction of reactive oxygen species (ROS), such as hydroperoxides, occurs due to repeated heating of Po. **Sedlak *et al.* (2009)** reported that heme oxygenase (HO), the key regulatory enzyme in heme breakdown, generates free ferrous ions, biliverdin, and carbon monoxide (CO). Biliverdin is subsequently transformed into bilirubin, which functions as an antioxidant. **Li *et al.* (2019)** found that heme oxygenase activity was significantly reduced in the heated Po groups suggesting a marked increase in oxidative stress as a result of repeated heating.

Li *et al.* (2017); Famurewa *et al.* (2017) reported that heated Po leads to an increase in liver function enzyme levels, extensive hepatocyte apoptosis, and the progression of chronic liver disease. **Morshed *et al.* (2018)** reported that studies on rats that were fed reheated Po revealed the presence of swollen hepatocytes, microgranules, and chronic inflammatory cells, though no hepatocyte necrosis was observed. **Venkata and Subramanyam (2016)** shown that there is a dose-dependent response to heated Po intake in terms of hepatocyte vacuolation. **Ani *et al.* (2018)** revealed that long-term consumption of thermally oxidized Po has been linked to thrombocytopenia, which results from bone marrow infiltration by adipose tissue and a decline in blood-forming cells. Additionally, prolonged consumption of oxidized Po leads to widespread hepatic steatosis (fatty liver) and hepatocellular necrosis. **Ani *et al.* (2018)** confirmed that diets supplemented with heated Po caused lipid droplets to accumulate, blood sinusoids and the central vein to dilate and become congested,

collagen to be deposited, a marked infiltration of periportal inflammatory cells, and the degeneration and necrosis of several hepatocytes, according to histological analysis of the liver in rats. **Chang *et al.* (2021)** demonstrated a hepatic cell swelling and necrosis by using mice fed on heated Po. **Taub (2004)** revealed that by encouraging hepatocyte proliferation and secreting hepatocyte growth factor (HGF), inflammation triggers hepatic stellate cells (HSCs) to start liver regeneration. Furthermore, the production of interleukin-6 (IL-6) by activated Kupffer cells promotes the hepatic expression of several genes linked to acute-phase proteins, redox balance, cell cycle regulation, and anti-apoptotic pathways. This helps the surviving hepatocytes proliferate. **Poisson *et al.* (2017)** revealed that hepatic sinusoidal reorganization after liver injury is significantly influenced by liver sinusoidal endothelial cells (LSECs). Furthermore, lysosomes can remove trash from degenerating particles, and the majority of liver cells have two nuclei, showing an extraordinary capacity for regeneration.

Ku *et al.* (2014) revealed that oxidative stress exerts widespread adverse effects across multiple physiological systems in the body. **Ighodaro and Akinloye (2018)** revealed that previous studies have investigated superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) as key biological markers of oxidative stress and lipid peroxidation. **Claudio *et al.* (2003);** reported that living organisms have evolved antioxidant defence systems to protect against oxidative stress-related damage. **Li *et al.* (2011)** reported that co-expression of SOD and CAT genes in *Lactobacillus rhamnosus* significantly enhanced oxidative stress resistance, suggesting a synergistic antioxidant mechanism. **Dimitrow *et al.* (1991)** showed that a rise in myocardial glutathione peroxidase (GPx) activity was noted in rats fed a lower dose of Po, while no further increase was observed with a higher dose. **Narsaria (2012)** demonstrated that rather than a decrease in antioxidants, oxidative stress seems to result from a surge in the production of free radicals, as evidenced by elevated levels of malondialdehyde (MDA), a crucial indicator of lipid peroxidation. **Khoschsorur *et al.* (2000)** demonstrated that lipid peroxidation was detected by measuring the amount of malondialdehyde (MDA) in the liver. The most common aldehyde produced during lipid peroxidation is malondialdehyde (MDA), which is frequently used as a proxy for oxidative stress. **Chong *et al.* (2019)** reported that this liver injury is attributed to the reduced antioxidant content of heated Po, along with the release of toxic compounds such as free fatty acids, aldehydes, ketones, alcohols, and reactive oxygen species (ROS). These compounds contribute to a decline in hepatic glutathione levels and initiate lipid peroxidation, leading to the production of malondialdehyde (MDA). The accumulation of MDA damages the hepatocyte membrane system, ultimately resulting in hepatocyte degeneration and necrosis. **Leong *et al.* (2009)** found that after giving rats regularly heated Po, their nitric oxide (NO) levels decreased.

Venkata and Subramanyam (2016) showed that the repeated use of frying Po presents considerable health risks, including histopathological alterations and modifications to genetic material. **Siddiq *et al.* (2019)** showed that during heating Po, lipid peroxidation generates free radicals that can compromise membrane lipids, potentially resulting in oxidative stress-mediated modifications to genetic material . **Ji *et al.* (2017)** indicated that prolonged heating duration increased free radical production in Po, reflecting greater oxidative deterioration and a heightened likelihood of DNA damage. **Kumar *et al.* (2024)** observed a higher frequency of micronuclei formation and chromosomal abnormalities in rats exposed to repeatedly heated oils, proposing that polycyclic aromatic hydrocarbons generated during prolonged heating may induce oxidative stress and DNA damage, ultimately concluding that long-term consumption of thermally degraded oils elevates health risks due to

genotoxic effects, with rats consuming five-time-heated oil showing greater DNA damage than those exposed to once-heated oil.

Narasimhamurthy and Raina (**2000**) reported that after 20 weeks of feeding rats thermally oxidized dietary oils, no appreciable histopathological changes were seen. The low levels of harmful substances produced during frying or the diet's macro- and micronutrient balance, which would have reduced possible toxicity, were the authors' explanations for this discovery. **Muharis *et al.*** (**2010**) reported that antioxidants with free radical scavenging properties in Po play a protective role in safeguarding endothelial cells from oxidative damage, thereby enhancing endothelial function .**Tan *et al.*** (**2018**) recommended that while Po oxidative stability makes it a suitable choice for cooking, caution should be exercised with repeated heating . **Meijaard *et al.*** (**2018**) raised environmental concerns regarding Po production, noting its significant role in deforestation and biodiversity loss.

3.Materials and methods

3.1 Animals

Male CD-1 albino mice (*Mus musculus*) weighing around 25 ± 5 g and 6–8 weeks of age were acquired from the Theodor Bilharz Research Institute in Cairo. When the mice arrived, they were placed in typical plastic cages and given a week to become acclimated to their surroundings before the study started. During this period, they were fed a typical pellet diet and had unlimited access to water. Under a 12-hour light/dark cycle, the temperature was kept at 25 ± 2°C and the relative humidity at 55 ± 10%. The Experimental Animal Care and Research Ethics Committee of Ain Shams University authorized all experimental methods, which were carried out in accordance with ethical standards.

3.2 Source and preparation of palm oil diets

The fresh palm oil (Po) was obtained from Rabie Abu Al-Ezz for Vegetable Oils, Zakat Foundation, Al-Shurafa Street, Cairo, Egypt. The Po used in this study is Refined Bleached Deodorized (RBD) Po, without any synthetic antioxidants added. It was produced by refining crude Po.

The Po was utilized in two forms: fresh oil and oil that had been heated once for ten minutes at 180°C directly over an open flame.

3.3 Experimental design

Thirty mice were randomly distributed into three groups of ten each. The groups were organized as follows: **Group 1** acted as the control, receiving 2.5 ml of distilled water five consecutive days each week for a total of two weeks. **Group 2** received an oral treatment of unheated Po at a dose of 2.5 ml every five days for two weeks. **Group 3** was treated orally with the same amount of heated Po 2.5 ml provided five days a week for two weeks. (Ilyas, 2018; 052032). Both the control and treated animals were euthanized by cervical dislocation 24 hours following the last treatment, and samples were taken for further examination.

3.4 Bodyweight

Each mouse's body weight was weighed using a digital scale at the beginning of the experiment and on the day of sacrificing.

The percentage of body weight change was evaluated by counting the difference between final weight and initial weight and divided by initial weight per each group (three animals per each group) and expressed in percentage.

3.5 Micronucleus test

With a few minor adjustments, the micronucleus test was carried out in accordance with Schmid's standard protocol (Schmid, 1976, 31–53). In particular, rather of using fetal calf serum as the suspending medium, 5% bovine albumin (purchased from the National Research Center, Giza, Egypt) was utilized (Narayana et al., 2002, 179-185).

The mice were killed at the end of the experiment, and their femurs were meticulously cut. The marrow cavity was then reached by inserting a blunt needle. To create a fine suspension, the bone marrow was flushed out using a syringe filled with 5% bovine albumin. After centrifuging this slurry for 8–10 minutes at 1000 rpm, the supernatant was disposed of. A Pasteur pipette was used to homogenize the mixture after a tiny amount of fresh suspending medium was introduced.

Three to four slides were prepared for each animal, and a drop of the prepared suspension was applied to one end of each slide to form a smear. The slides were fixed with methanol for five minutes after being allowed to air-dry for the entire night. Giemsa and phosphate buffer (pH 6.8) were used to stain them after drying so that micronuclei could be seen. The slides were thoroughly cleaned using buffer and distilled water, then dried and mounted. Each animal had 2000 PCEs analysed, and the quantity of micronucleated PCEs (MNPCEs) was noted. The normochromatic erythrocytes (NCEs) that were found were also recorded. For every animal, the PCE/NCE ratio and the percentage of MNPCEs were computed.

3.6 Histological Examination

Following excision, little samples of liver tissue were fixed for the night in 10% neutral-buffered formalin after being promptly cleaned with phosphate-buffered saline. Following a sequence of graded ethanol dehydration, the tissues were embedded in paraffin. Using a Microm HM315 microtome (Thermo Scientific, Walldorf, Germany), paraffin-embedded materials were cut into slices that were 5 μm thick and placed on glass slides. Hematoxylin and eosin (H&E) staining was applied to the sections, and an Axiostar Plus microscope (Carl Zeiss, Oberkochen, Germany) was used to view them at a 400× magnification. An EOS SDS digital camera and EOS software (Canon®, Tokyo, Japan) were used for image analysis.

3.7 Oxidative stress marker analysis

3.7.1 Determination of Malondialdehyde (MDA)Concentration in plasm

Malondialdehyde (MDA) concentrations were determined using the Gutteridge and Wilkins method (Gutteridge et al., 1982, 327–329). The $(TBA)_2$–MDA adduct, which absorbs at 532 nm, is the pink chromogen that is formed when MDA reacts with thiobarbituric acid (TBA) in an acidic and heated environment. Two millilitres of glacial acetic acid, two millilitres of 1% TBA, and 0.2 millilitres of plasma were combined for the operation. Following 15 minutes of heating in boiling water with sporadic spinning, the mixture was loosely stoppered, cooled, and centrifuged at 5000g for 10 minutes at room temperature. In comparison to a reagent blank, the absorbance of the resultant supernatant was measured at 532 nm. The molar extinction coefficient of 1.56×10^5 L/mol/cm was used to compute MDA concentrations, which were then represented as moles of MDA per litre of plasma.

3.7.2 Superoxide (SOD) Activity

The colorimetric approach of the Superoxide Dismutase (SOD) assay is based on the enzyme's capacity to prevent the reduction of nitro blue tetrazolium (NBT) by phenazine methosulfate (PMS). PMS, NBT, NADH, and phosphate buffer (pH 8.5) were combined to create the working reagent. A diluted sample was added to the reaction mixture for the experiment, and absorbance at 560 nm was measured for five minutes at 25°C.By comparing the absorbance change with and without the material, the percent inhibition was determined. Based on the proportion of inhibition, the enzyme activity was expressed in units per millilitre, gram of tissue, or gram of hemoglobin. Accurate results were guaranteed by careful sample preparation, which included dilution and removal of redox-active substances.

3.7.3 Catalase (CAT) Activity

A colorimetric technique for measuring catalase activity based on its capacity to break down hydrogen peroxide ($H2O_2$) is the catalase (CAT) assay. The sample was added to a solution containing $H2O_2$ to start the reaction, and a catalase inhibitor was added to stop it after a minute.

A quinoneimine dye was subsequently created by the reaction of the residual H_2O_2 with peroxidase (HRP), 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS), and 4-aminophenazone (AAP). At 510 nm, the dye's intensity was inversely correlated with the catalase activity. Units per gram (U/g) for tissue or units per litre (U/L) for plasma were used to express enzyme activity. After being prepared by centrifugation and homogenization, the samples were kept for subsequent examination at -80°C.

3.7.4 Glutathione Peroxidase (GPx) Activity

The oxidation of NADPH to $NADP^+$, which is accompanied by a drop in absorbance at 340 nm, is the basis for the glutathione peroxidase (GPx) assay, which quantifies enzyme activity. In order to start the process, hydrogen peroxide was added after a sample was mixed with phosphate buffer, glutathione (GSH), glutathione reductase,

and NADPH. Over the course of three minutes, the rate of NADPH oxidation was measured spectrophotometrically and was directly proportional to the sample's (GPx) activity. After accounting for sample dilution, the change in absorbance was converted to NADPH consumption (nmol/min/mL) to determine the enzyme activity. The sample was prepared by centrifuging it to produce a clear supernatant and homogenizing it in a phosphate buffer with reducing agents. The stability of (GPx) activity for precise analysis was guaranteed by appropriate handling and storage at -70°C.

3.8 Data analysis

Software called SPSS (version 16.0) was used to conduct statistical analysis. The mean ± standard deviation (mean ± SD) was used to express the data. To assess whether differences between the two groups were statistically significant or the result of sampling error, an independent samples t-test was used. A statistically significant level was defined as $P < 0.05$, but a higher degree of significance was denoted by $P < 0.01$. On the other hand, differences were considered non-significant if $P > 0.05$. Excel 2019 was used to create the visual representations.

4. Results

4.1 Body Weight Changes

As shown in the **figure** (**1**), the percentage increase in body weight in the control group was 25.2 ± 2.8%. Group 2 exhibited a statistically significant increase in body weight (27.9 ± 1.5%) compared to the control group. Conversely, Group 3 demonstrated a statically significant reduction in body weight gain (1.74 ± 0.49%) relative to the control. These results suggest that fresh palm oil may promote body weight gain, whereas repeated heating of Po markedly impairs this effect, potentially due to the breakdown of oil condition or the creation of dangerous substances during the heating process, thereby indicating possible adverse metabolic impacts.

Figure (1): Percentages of changes between initial and final body weights in the control and treated group.

4.2 Micronucleus test

The micronucleus test findings are shown in **Table** (**1**). Normochromatic erythrocytes (NCEs) had a dark blue stain, as seen in **Figure** (**2**), whereas polychromatic erythrocytes (PCEs) had staining that ranged from light blue to violet. Polychromatic erythrocytes with one or more tiny nuclei that had a dark blue, ring-shaped appearance were known as micro-nucleated polychromatic erythrocytes (MNPCEs). Acentric chromosomal fragments that do not adhere to the spindle during the anaphase phase of cell division are indicated by this trait. The results of the micronucleus assay showed clear evidence of genotoxicity and cytotoxicity in mice treated with both fresh and heated palm oil when compared to the control group. In the control group, the frequency of micro-nucleated polychromatic erythrocytes (MNPCEs) was low (0.90 ± 0.13), indicating normal cellular conditions with minimal DNA damage, and the PCE/NCE ratio was close to 1 (1.01 ± 0.01), reflecting a healthy balance between immature and mature erythrocytes in the bone marrow. However, treatment with fresh Po led to a substantial increase in MNPCEs (5.73 ± 1.21), suggesting increased DNA damage. Additionally, the PCE/NCE ratio rose to 1.53 ± 0.42, indicating a moderate level of cytotoxicity, possibly due to stress on the bone marrow that altered normal erythropoiesis. In comparison, the group treated with heated Po exhibited the most severe effects, with the highest frequency of MNPCEs (8.16 ± 0.41), strongly indicating significant genotoxic stress. Furthermore, the PCE/NCE ratio in this group dramatically increased to 4.97 ± 2.63, This indicates a significant degree of cytotoxicity, most likely brought on by harmful byproducts created when the oil was heated repeatedly. In comparison to the control, both treated groups had a statistically significant increase in bone marrow toxicity and DNA damage overall, with heated Po having more detrimental effects than fresh Po.

Groups	Total scored cells/ No. of mice	Total MNPCEs	Micronuclei MNPCEs/Total PCEs % (Mean± SD)	Total NCEs	Cytotoxicity PCEs/ NCEs (Mean± SD)
Group 1 (Control)	6000/3	54	0.9000± 0.13229	5955	1.0074± 0.00673
Group 2 (Fresh palm oil treated-group)	6000/3	344	5.7333± 1.20554*	4120	1.5270± 0.42158*
Group 3 (Heated palm oil treated- group)	6000/3	486	8.1600± 0.41012*	2240	4.9667± 2.62968

Table (**1**) **represents the mean and standard deviation of micro-nucleated polychromatic erythrocytes** (**MNPCEs**) **and PCEs/NCEs ratio in 6000 polychromatic erythrocytes** (**PCEs**) **and corresponding normochromatic erythrocytes** (**NCEs**) **scored in the bone marrow of albino mice of the control group and treated groups**.

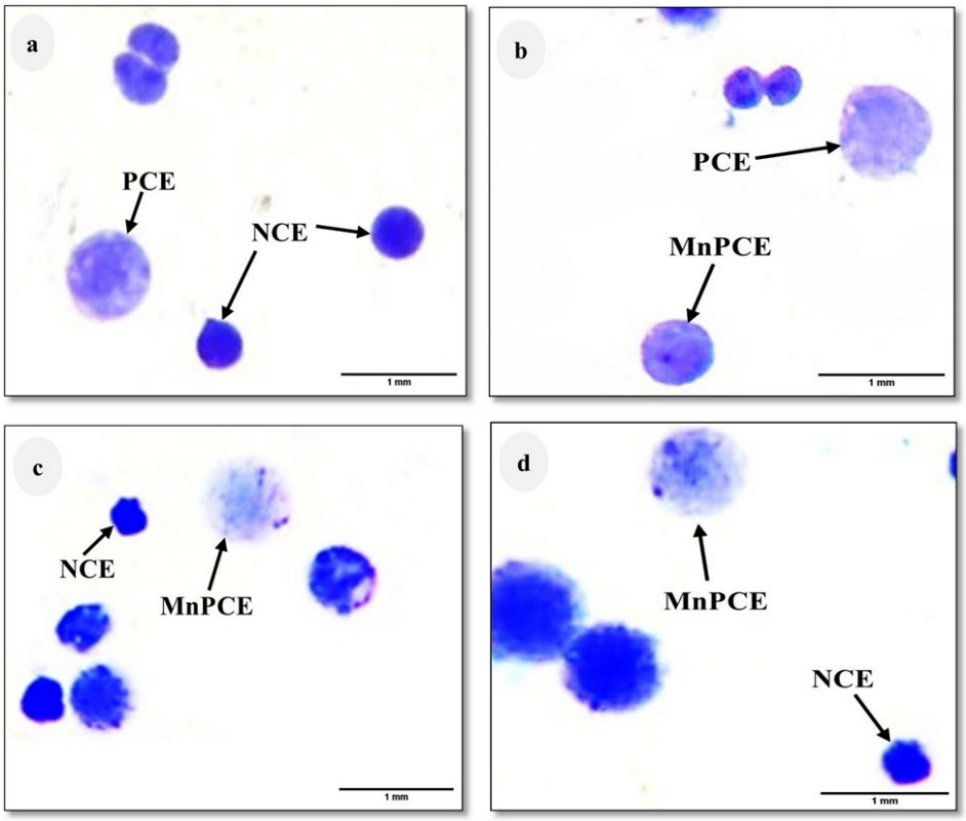

Figure (2) Bone marrow smears of Mus musculus showing polychromatic erythrocytes (PCE), normochromatic erythrocytes (NCE), and micro-nucleated polychromatic erythrocytes (MnPCE). (a) Control group, (b) Group 2, (c and d) Group 3. The scale bar is 0.2 mm.

4.3 Histological and histopathological observations

Histological examination of liver tissues stained with hematoxylin and eosin (H&E) revealed significant structural differences among the experimental groups. As illustrated in **Figure 3**, the control group exhibited normal hepatic architecture, with hepatocytes displaying abundant cytoplasm and small, centrally located nuclei. The hepatic cords were orderly, divided by thin-walled blood sinusoids, and a normal central vein was noticed. In contrast, the group treated with fresh palm oil (**Figure 4**) showed clear signs of pathological alterations, including fatty changes in the hepatic tissue, distention of the hepatic sinusoids, and irregularity in the arrangement of hepatic cords. These findings indicate early signs of liver stress due to the intake of fresh Po. More severe histological alterations were observed in the group treated with heated Po, as shown in **Figure 5**. This group exhibited necrosis of hepatocytes, infiltration of inflammatory cells, and the presence of fat globules within some hepatocytes, suggesting significant liver damage possibly induced by oxidative stress associated with repeated heating of the oil.

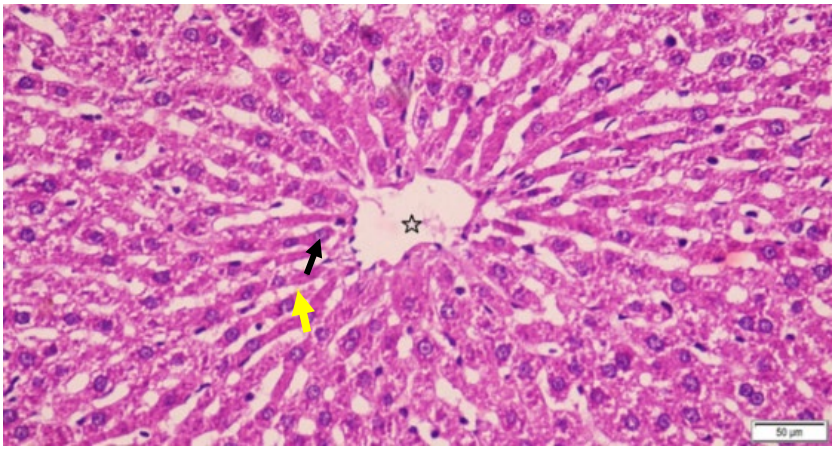

Figure (3): Normal histological structure of liver sections of albino mice: Photomicrographs sections of liver stained with hematoxylin and eosin (H&E) illustrate pathological changes. This group (control) showing normal hepatocytes with abundant cytoplasm and small nuclei (black arrow) and separated by thin wall blood sinusoids (yellow arrow) with normal central vein (black star). (Scale bar = 50 µm).

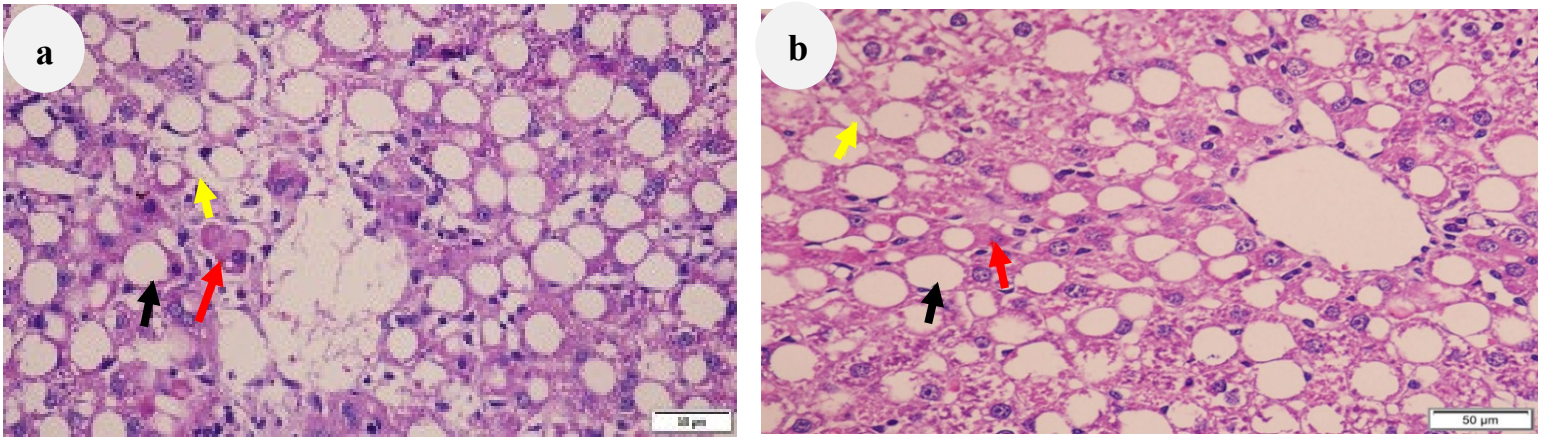

Figure (4): Histological alterations in liver tissue of mice: Photomicrographs of sections of liver stained with hematoxylin and eosin (H&E) illustrate pathological changes. This group treated with fresh palm oil, in panel (a and b), showing fatty change of hepatic tissue (black arrow), distention of hepatic sinusoids (yellow arrow) and irregularity of hepatic cords (red arrow). (Scale bar = 50 µm).

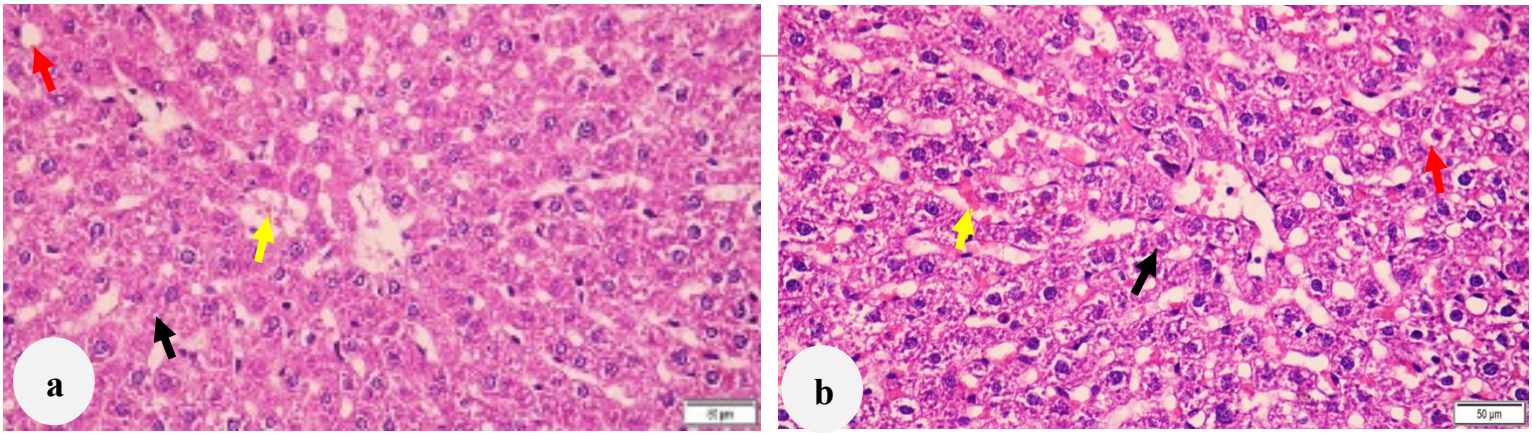

Figure (5): Histological alterations in liver tissue of mice: Photomicrographs of sections of liver stained with hematoxylin and eosin (H&E) illustrate pathological changes. This group treated with heated palm oil, in panel (a and b), showing Necrosis of hepatocytes (black arrow), inflammatory cells infiltration (yellow arrow) and fat globule of some hepatocytes (red arrow). (Scale bar = 50 µm)

4.4 Oxidative stress markers

The present study looked into the impact of fresh and heated palm oil treatment on oxidative stress markers in liver tissues of albino mice (*Mus musculus*). The evaluated parameters included malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) across control and treated groups. All results are shown as mean ± SD, and $P < 0.05$ and $P < 0.001$ were regarded as statistically significant.

Current results showed statistical differences in the levels of MDA, SOD, CAT and GPx., a statistically significant increase in MDA levels in the treated groups compared to the control group, suggesting elevated lipid peroxidation and oxidative damage in renal tissues. In contrast, the actions of key antioxidant enzymes, (SOD), (CAT), (GPx) were significantly reduced in treated groups relative to the control, indicating impairment of the antioxidant defense system.

4.1 Malondialdehyde (MDA) Levels

The concentration of malondialdehyde (MDA) in liver tissues was significantly elevated in Group 2 (1.8433 ± 0.08021 mol/mg protein) and Group 3 (2.6933 ± 0.40104 nmol/mg protein), in contrast to the control group to the control group (0.8533 ± 0.02517nmol/mg protein) (**Figure 6**). This increase in MDA levels indicates enhanced lipid peroxidation and suggests the presence of oxidative stress in treated groups.

4.2 Superoxide Dismutase (SOD) Activities

Superoxide dismutase (SOD) activity was significantly reduced in the treated groups in contrast to the control. Group 2 demonstrated a highly significant decrease (3.3367 ± 0.09452 U/mg protein), while Group 3 showed a further significant reduction (1.9700 ± 0.09849 U/mg protein), relative to the control group

(4.4333 ± 0.06506 U/mg protein) (**Figure 7**). These findings suggest impaired enzymatic defense against superoxide radicals following treatment.

4.3 Catalase (CAT) Activity

Catalase (CAT) activity was greatly reduced in the treated groups in contrast to the control. Group 2 exhibited a reduction in CAT activity (2.6767 ± 0.18583 U/mg protein), while Group 3 demonstrated a further significant decline (1.0100 ± 0.07211 U/mg protein), relative to the control group (3.6200 ± 0.09539 U/mg protein) (**Figure 8**). This reduction reflects diminished enzymatic capacity to neutralize hydrogen peroxide under oxidative stress conditions.

4.4 Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase (GPx) activity was significantly ($P < 0.05$) decreased in the treated groups compared to the control. Group 2 exhibited a highly significant ($P < 0.001$) reduction in GPx activity (4.6767 ± 0.15011 U/mg protein), while Group 3 showed a more pronounced decrease (2.4433 ± 0.15822 U/mg protein), relative to the control group (6.5000 ± 0.19975 U/mg protein) (**Figure 9**). These findings indicate a substantial impairment in the enzymatic detoxification of peroxides in response to treatment.

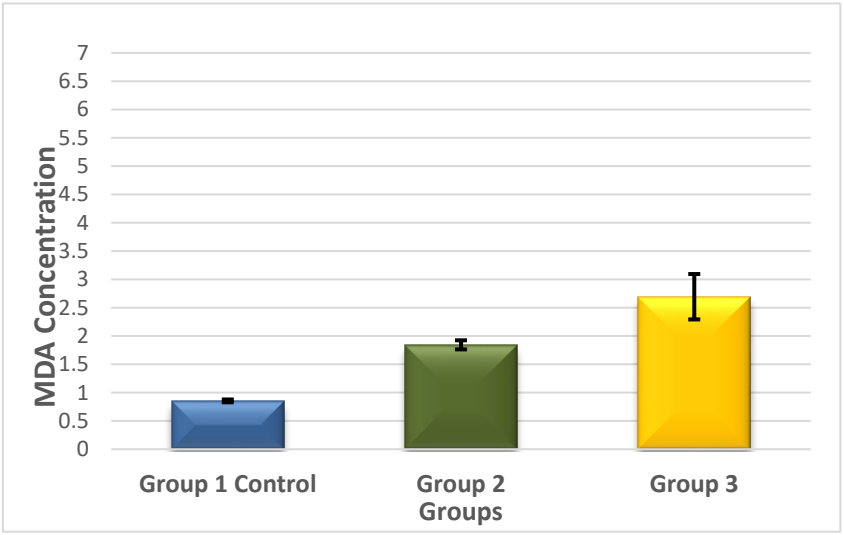

Figure (6): Malondialdehyde (MDA) level in liver of albino mice *Mus Musculus*, (mean ± SD). Malondialdehyde (MDA) level expressed as unit nmol mg^{-1} protein.

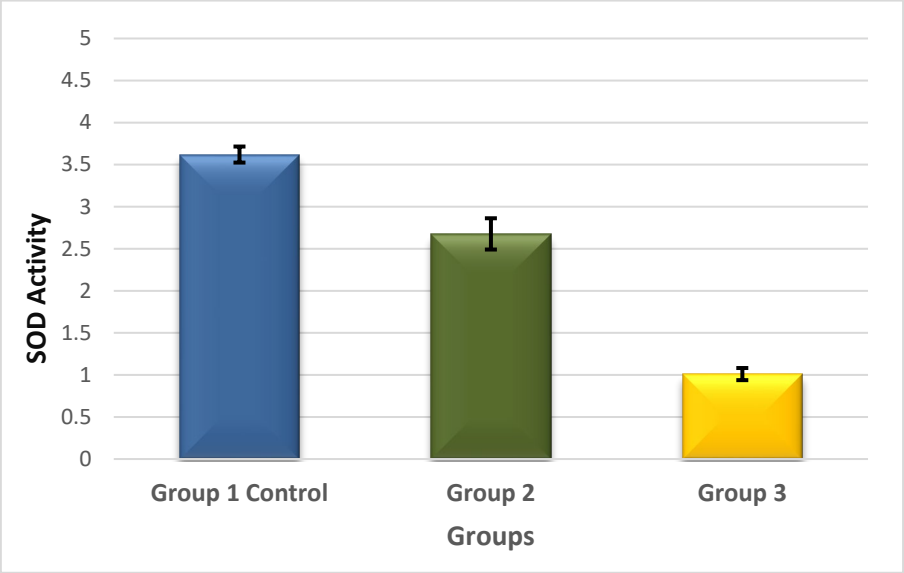

Figure (7): Superoxide dismutase (SOD) activity in liver of albino mice *Mus Musculus*, (mean ± SD). Superoxide dismutase activity is expressed as unit U mg^{-1} protein.

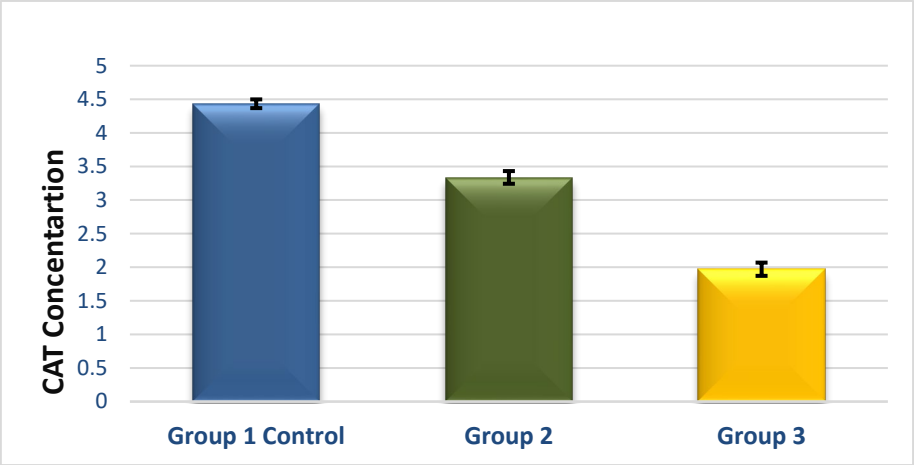

Figure (8): catalase (CAT) activity in liver of albino mice *Mus Musculus*, (mean ± SD). Superoxide dismutase activity is expressed as unit U mg^{-1} protein.

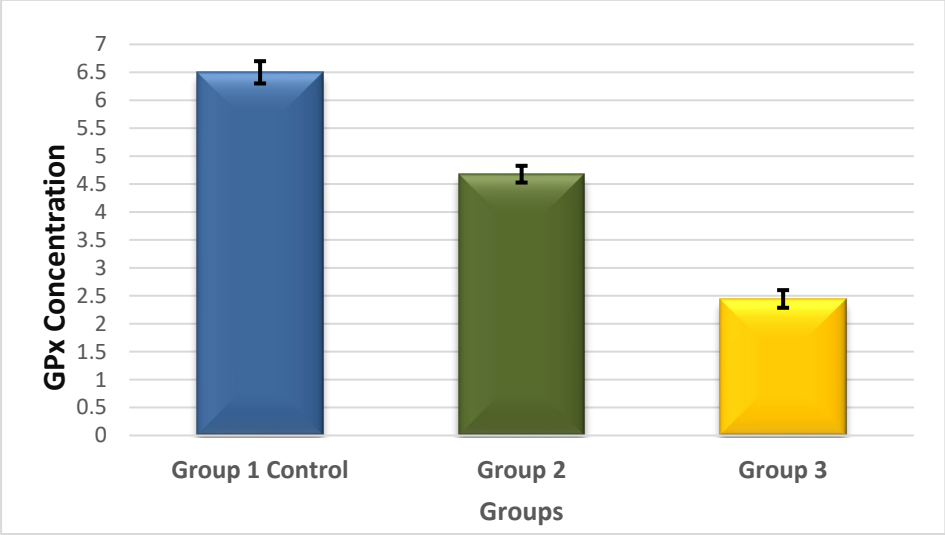

Figure (9): Glutathione Peroxidase (GPX) activity in liver of albino mice *Mus Musculus*, (mean ± SD). Superoxide dismutase activity is expressed as unit U mg^{-1} protein.

5. Discussion

Palm oil derived from the mesocarp of oil palm fruits, is a versatile edible oil utilized in various applications, including food production, cosmetics, and bioenergy generation. Po is distinct owing to its balanced ratio of fatty acids with a near-equimolar proportion of saturated fatty acids (SFAs) to unsaturated fatty acids (USFAs). Additionally, it contains a high amount of vitamin E, a potent antioxidant capable of scavenging free radicals and potentially mitigating oxidative stress. Po is a commonly utilized edible oil in everyday culinary practices, serving as a prevalent ingredient in various food preparations. Frying is a widely employed and enduring method of food preparation, remaining a prevalent culinary practice globally. Repeated heating of Po resulted in an initial increase followed by a decrease in unsaturated fatty acid (USFA) content, whereas saturated fatty acid (SFA) levels remained relatively higher. This alteration in fatty acid composition is consistent with previous findings (Leong *et al.,* 2012, 20-29), highlighting the detrimental effects of high-temperature heating on oil quality. Thermal degradation of vitamin E occurs with repeated heating of frying Po, consistent with prior research (Andrikopoulos *et al.,* 2002, 37-177; Leong *et al.,* 2008,567–572). Body weight was included as a key parameter in this study because it serves as a general indicator of systemic toxicity and nutritional status; fluctuations in weight can reflect metabolic disturbances induced by the consumption of palm oil after repeated heating. The present study indicates that intake of fresh Po is associated with a notable increase in body weight, whereas repeated heating of palm oil significantly diminishes this effect. This observation aligns with previous research demonstrating that thermal oxidation of Po alters its physicochemical properties, leading to the formation of harmful compounds that may adversely affect health. For instance, heating palm oil increases its peroxide value, indicating lipid peroxidation and degradation of oil quality (Akinola *et al.,* 2010,781- 784). Similarly, chronic intake of thermally oxidized Po resulted in reduced weight gain and unfavourable lipid profile changes in rats (Owu *et al.*, 2005, 93–98). Consumption of oxidized Po led to elevated blood pressure and cholesterol levels,

suggesting potential cardiovascular risks (Ebong *et al.,* 1999, 341-345). Furthermore, while fresh Po consumption contributed to weight gain, the heated variant exhibited opposite effects (Oguntibeju *et al.,* 2009, 4683–4687.). Bone marrow is commonly investigated in studies involving heated palm oil due to its sensitivity to oxidative stress and its critical role in hematopoiesis, positioning it as a crucial marker of systemic toxicity and highlighting the possible effects of oxidized dietary lipids. The findings of the present study are in strong agreement with the observations of (Kumar *et al.,* 2024, 45-55), who reported a dose-dependent increase in micronuclei formation and chromosomal abnormalities in mice exposed to repeatedly heated Po. Our micronucleus test revealed that both fresh and heated Po induced significant genotoxicity and cytotoxicity in mice, with heated Po exhibiting the most severe effects mirroring Kumar et al.'s conclusion that thermal degradation amplifies DNA damage These results align with the observations of (Mesembe *et al,* 2004, 86-91), who reported a significant reduction in red blood cell (RBC) count in mice fed a thermally oxidized Po diet compared to those fed fresh Po or control diets. This reduction was attributed to the suppressive effects of toxic compounds formed during oil oxidation on bone marrow function. Similarly, in the present study, the heated Po group exhibited the highest DNA damage and severe cytotoxicity, further supporting the detrimental impact of oxidized Po on hematopoietic tissues. The liver was chosen as a target organ in this study because of its vital function in metabolizing dietary fats and its high susceptibility to oxidative damage caused by toxic lipid peroxidation products formed during the repeated heating of palm oil. Results of the current study showed that histopathological analysis revealed pronounced hepatic damage in mice fed heated Po, contrasting with the control group, which displayed typical liver histology, including organized hepatic cords, normal hepatocyte morphology, and intact sinusoidal structure, consistent with standard hepatic histology as confirmed in this study (Mescher, 2021). Mice fed fresh Po exhibited early hepatic abnormalities including steatosis, sinusoidal dilation, and disrupted hepatic cord architecture. Steatosis, a key indicator of impaired lipid metabolism, is frequently a prelude to NAFLD, or non-alcoholic fatty liver disease. particularly in the context of saturated fat-rich diets (Schenk *et al.*, 2008,2992–3002). Hepatic sinusoidal dilation observed in this study likely reflects underlying oxidative stress or endothelial dysfunction, consistent with prior reports of early liver injury models. Mice exposed to heated Po exhibited the most pronounced hepatic damage, characterized by extensive hepatocyte necrosis, inflammatory cell infiltration, vacuolar degeneration, and marked steatosis. Aldehydes and other hazardous lipid peroxidation products are produced, likely contributed to the observed hepatic inflammation, apoptosis, and oxidative damage, consistent with previous findings (Chong *et al.*, 2019, 989–1000). Hepatocellular necrosis and vacuolar degeneration indicate irreversible liver damage, potentially resulting from mitochondrial dysfunction and lipid peroxidation-mediated membrane damage, consistent with established mechanisms of cellular injury (Pizzino *et al.*, 2017). Hepatic histopathology in heated Po-treated groups revealed pronounced hepatocyte degeneration, compromised cellular integrity, and hydropic changes, aligning with prior studies demonstrating similar hepatic alterations (Shastry *et al.*, 2011, 10–15; Morshed et al., 2018, 96). Hepatocyte vacuolation was observed in response to heated Po consumption, consistent with previous findings of dose-dependent vacuolation (Venkata *et al.,* 2016, 636–43). Oxidative stress markers are essential indicators in evaluating the toxic effects of fresh and heated palm oil. The current study shows that oxidative

stress markers in the liver tissues of mice are significantly impacted by both fresh and repeatedly heated Po. A significant elevation in MDA levels in the treated groups, particularly in group treated with heated Po, indicates an increase in lipid peroxidation, suggesting oxidative damage due to the generation of reactive oxygen species (ROS). This is in agreement with the findings of (Chow *et al.*, 2010, 936-942) elevated MDA levels following consumption of oxidized Po. Furthermore, the activities of antioxidant enzymes: SOD, CAT, and GPx were significantly decreased in treated groups. This reduction suggests an overwhelmed or impaired antioxidant defence mechanism. These results are in line with a study that shown that mice's hepatic antioxidant enzyme activity decreased when they consumed thermally oxidized Po. (Owu *et al.*, 2005, 036–041). Similarly, repeated heating of Po causes the formation of polar compounds and free radicals, which may deplete endogenous antioxidant reserves (Sundram *et al.*, 2003, 355-362). The greater oxidative Imbalance in group with heated Po compared to Group treated with fresh Po suggests a dose- or frequency-dependent effect of heating on oil toxicity. Heating repeatedly seems to promote the production of harmful substances such polymers, aldehydes, and hydroperoxides, which worsen oxidative stress. These oxidative by-products from oil degradation can alter cell membrane integrity and enzyme activity (Choe *et al.,* 2007, 77-86).

6. Conclusion

The results of this research highlight the harmful impact of both fresh and heated palm oil, as evidenced by liver tissue damage, increased oxidative stress, reduced antioxidant enzyme activity, and genotoxicity observed through the micronucleus test in mice. These results indicate that thermal oxidation significantly compromises the safety of palm oil and may lead to serious health risks. Therefore, it is recommended that individuals avoid reusing palm oil for cooking purposes. It is essential to increase public awareness regarding the specific health hazards associated with the consumption of thermally processed palm oil. Promoting healthier substitutes, including olive oil, canola oil, and sunflower oil, may encourage improved dietary habits and contribute to the reduction of long-term health risks.

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Abbreviations

Po: palm oil
MDA: malondialdehyde
SOD: superoxide dismutase
CAT: catalase
GPx: glutathione peroxidase

المستخلص العربي

زيت النخيل يُستخدم بشكل شائع في الطهي، إلا أن تسخينه المتكرر قد يؤدي إلى تغيرات كيميائية ضارة. تهدف هذه الدراسة إلى التحقيق في الآثار السلبية لكل من زيت النخيل الطازج والمسخن على الإجهاد التأكسدي، والتغيرات النسيجية في الكبد، والتسمم الجيني باستخدام نموذج حي من الفئران. تم تقسيم ثلاثين فأرًا أبيض من نوع (*Mus musculus*) CD-1 إلى ثلاث مجموعات تجريبية بشكل متساوٍ: مجموعة ضابطة لم تتلقَ أي علاج، مجموعة عولجت بزيت النخيل الطازج، ومجموعة عولجت بزيت النخيل المسخن لمدة خمسة أيام متتالية في الأسبوع على مدار أسبوعين. أظهرت قياسات الوزن اختلافات واضحة بين المجموعات، حيث سجلت مجموعة زيت النخيل الطازج أعلى زيادة في الوزن (27.9%) مقارنة بالمجموعة الضابطة (25.2%)، في حين أظهرت مجموعة زيت النخيل المسخن نمواً ضعيفاً جداً (1.74%). وأكد اختبار النواة الصغيرة في خلايا نخاع العظم وجود تأثير سام للجينات ناتج عن زيت النخيل المؤكسد حراريًا. أما الفحص النسيجي لأنسجة الكبد فقد أظهر بنية كبدية سليمة في المجموعة الضابطة، في حين لوحظت تغيرات طفيفة مثل التنكس الدهني وتوسع الجيوب الدموية في مجموعة زيت النخيل الطازج. في المقابل، أظهرت مجموعة زيت النخيل المسخن تلفًا كبديًا شديدًا تميز بوجود نخر واسع، وتسربات التهابية، وتنكس دهني واضح، واحتقان وعائي. كما كشفت التحاليل الكيميائية الحيوية عن ارتفاع كبير في مستويات المالوندي ألدهيد في مجموعة الزيت المسخن، مصحوباً بانخفاض ملحوظ في أنشطة إنزيمات مضادة للأكسدة مثل السوبر أكسيد ديسميوتاز، والكاتاليز ، والجلوتاثيون بيروكسيداز، مما يشير إلى تحفيز قوي للإجهاد التأكسدي. وتؤكد هذه النتائج أن زيت النخيل الطازج قد يساهم في زيادة الوزن مع تأثيرات كبدية محدودة، في حين أن زيت النخيل المسخن يسبب أضرارًا تأكسدية شديدة، وتسممًا كبديًا، وأضرارًا في الحمض النووي، مما يُبرز المخاطر الصحية المرتبطة باستخدام زيت النخيل المسخن بشكل متكرر في الطهي. وبناءً على هذه النتائج، نوصي بشدة بتجنب استخدام زيت النخيل المسخن مرارًا وتكرارًا في ممارسات الطهي لما له من مخاطر صحية محتملة. وبدلاً من ذلك، يُنصح باعتماد زيوت طهي بديلة وأكثر أمانًا لتقليل التعرض للمركبات الضارة وتعزيز الصحة العامة.