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Alterations in Lipid biomarkers Induced by Re-used Oxidative Edible Palm Oil in Albino Mice (*Mus musculus Linnaeus* 1758

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In an attempt to explore the harmful effect of oxidative edible palm oil on body mass, cardiac alterations, and lipid profile in albino mice, an experiment using 24 mice was carried out divided into groups I, II, III, and fed with a normal diet, fresh and reused oxidized palm respectively. The result from the study showed that the mice in groups II and III indicated a considerable proliferation ($p<0.05$) within body mass. The total cholesterol of control (group I) were (54.12 ± 2.15 U/L), in fresh palm oil (group II) mice were (69.05 ± 8.53 U/L) while in reused oxidized oils show fundamentally influences in lipid profile. Cholesterol levels were (89.37 ± 8.26 U/L) Confirmed increase ($p<0.05$) levels of Cholesterol. Triglyceride (U/L) Level in (Group 1) control was (21.12±1.25U/L) in (Group II) fresh palm feed was (39.8750 ± 5.33U/L) and in (Group C) reused oxidized palm oil feed was (56.50 ± 5.24U/L) demonstrated major rise ($p<0.05$) in triglyceride. HDL levels in (Group I) control were (82.01 ±0.31 U/L).in (group II) fresh palm oil feed were (82.45 ± 4.01 U/L) while in (Group III) reused oxidized palm oil feed was (105.97±3.31 U/L). The group (III) demonstrated a noteworthy increase ($p<0.05$) in the HDL level. LDL levels in (Group I) control were (82.01 ±0.31 U/L).in (group II) fresh palm oil feed were (82.45 ± 4.01 U/L) while in (Group III) reused oxidized palm oil feed was (105.97±3.31 U/L). The group (III) demonstrated a noteworthy increase ($p<0.05$) in the LDL level. The histological reading demonstrated that the cardiac cells of the reused oxidized palm oil feeding group demonstrated vacuole and congested myocardial condition in papillary in heart muscles. The reused oxidized palm oil has adverse effects and should be discouraged.

Keywords: oxidized palm oil; lipid profile, low density proteins, histology

INTRODUCTION

Palm oil is an edible vegetable oil derived from both palm fruit pulp and seeds (Rossel, 1983).which is tropical monocotyledon perennial palm tree belonging to *Elaeis guineensis* (Pereira et al. 1990). (Rossel,1983; Gapor et al.1989). The

yellow color of palm *E. guineensis*, fruits are associated with ripening. The immature mesocarp contains large amount of chlorophyll and less carotene then ripens fruit (Khan et al. 2020). Chemically palm oil is highly saturated with fatty acids, about 50% esterified with glycerol (Leong, et al. 2015). Palm oil is semisolid at room temperature (Nor and Yusoff 2000). Like other vegetable oil, it is also the predominant normal source of the precursor of vitamin E to cotrienol and to copherols, also contain vitamin K and dietary magnesium with 10% linoleic acid source of omega 6 fatty acid (Gapor et al., 1989). Linoleic acid is very much essential for lowering cholesterol levels and also an anti-cancer agent. Tocotrienols are isomers of fat-soluble vitamin E and the main antioxidants component of palm oils (Yamada,et al. 2000).

Tocotrienols are antioxidants and are also essential for the body's defense mechanism against free radicals (Esterbauer et al., 1991). β-carotene is the most abundant carotenoid present in palm oil which is the main precursor of vitamin A (Gapor et al., 1989) vitamin A is very important in the optical process(Leong, et al. 2010). In addition, it is also an antioxidant that inhibits single oxygen in the body and cancer cells (Edem, 2002). Palm oil is edible oil used as both fresh and thermally oxidized form from ancient times (Rossel, 1983).

Generally, edible oils are oxidized to keep them more pleasant (Tappel et al. 1973). However, the long term thermally Oxidation impacts decreasing dietary oils quality (Isong, 1992). Also ingestion of thermally oxidized oil, there is accompanying by-product growth which is very cytotoxic and damaging (Pantzaris, 1995,) which are very deleterious to cells, tissues, and organs (Tappel 1973).

It has been speculated that absorption of oxidized oils to reduce critical fatty acid deficiency (Osim et al. 1992), fatty livers, growth retardation, atherosclerosis (Izaki et al. 1984). Malnourishment of micronutrients and the nucleic acid deficiency caused denaturing of enzymes (Hill et al. 1982). As thermally oxidation of palm oil and fatty acid produced different free radicals in a body (Isong et al. 1992) which may cause many diseases such as arthritis, cancer, cataracts formation, and diabetes (Pantzaris, 1995).

Therefore the present exploration is proposed to scrutinize the effects of reused edible palm oil on lipid profile and histological cardiac alteration in albino mice and to conduct histopathology to counter confirm the effect if any.

MATERIALS AND METHODS

Chemicals

Bio-Chemical Kits were used for the estimation or the counting of; total Cholesterol, triglyceride, HDL, LDL, and Histopathology of heart will be conducts.

Sample oils

In the present research The Palm, oil was used was divided into 2 groups at the ratio of 1:1 the fresh palm oil was feed to albino mice. While the remaining fresh palm oil was thermally oxidized for 10 hours at 100 °C in steeliness steel and was feed to albino mice at one gm. /kg body- weight dose. Animals use 24 albino mice, were used and kept in the stainless cage for adimitizataion with commercial albino mice, food and water was provided at libitum in animal house.

Experimental design

The 24 Albino mice, were divided into 3 groups each group comprised 8 mice. Oils sample were given to each group through oral route with following schedule:

Group I: Control (Normal food and clean water)

Group II: Fresh palm oil (FPO) at the dose rate of 1gm/kg

Group III: Thermally Oxidized palm oil (TPO) at dose rate of 1gm/kg

Albino mice were weighted twice, pre, and post of an experiment. Before sacrifice, blood was collected in a test tube without anticoagulant from the jugular vein, for biochemical analysis and estimation of Total Cholesterol (TC), Triglyceride (TG) HDL, and LDL. The heart, tissue was taken and preserved in formalin for histological examination (Godka, 1994; Osim et al. 1992).

Data analysis

Data are presented in Mean ± stander deviation by using software Statically Program of Social Science (SPSS). Values given in columns in results are significantly $p<0.05$.

RESULTS

To probe out the effects of oxidized palm oil on laboratory rearing albino mice, (*Mus. Musculus)* Linnaeus 1758.The adverse effect revealed an alteration in physicochemical characteristics of oxidized oils. The albino mice, which were feeds with fresh and thermally oxidized palm oil showed a significant increment in body weight. The histological study demonstrated the alteration in the tissue of heart cells in reused palm oil feed (group III).

Effects of reused palm oil in body weight of the albino mice Mus musculus Linnaeus 1758

During the present work the body weight in albin mice, (*M. musculus Linnaeus 1758)* were observed. The value of initial body weight in the (Group 1) control was (244 ± 4.95 g), (Group 2) fresh palm oil (243 ± 16.05g), and (Group 3) thermally oxidized palm oil feed were (233 ± 23.90 g) wt/g. whereas in the case of final body weight (Group 1) control were (268 ± 2.9g) (Group 2) fresh palm oil (296 ± 12.01g) and (Group 3) reused oxidized palm oil (297 ± 13.93g) wt/g. While in the case of weight gain the (Group 1) control value was (24 ± -2.86g), (Group 2) fresh palm oil (53.5 ± 4.04 g), and the value of (Group 3) reused oxidized palm oil was (64.11± 9.97g). The mice (Group 2) fed with (FPO) and (Group 3) feed reused palm oil indicated considerably increase ($p<0.05$) in weight compare with (Group 1) controlled during the study period (Table=1)

Effects of reused palm oil on total Cholesterol level of the albino mice, *M. musculus* Linnaeus 1758

The lipid profile of mice indicated a noteworthy increment in cholesterol level in every one of the group when contrasted with control (Group I) Total cholesterol levels in (Groups1) controlled were (131.65 ± 12.87mg/dl). In (Group II) fresh palm oil feeding was (252.33 ± 22.1mg/dl) while in (Group III) thermally oxidized was (311.23 ± 30.79 mg/dl). Increment in all-out cholesterol level of (Group 3) thermally-oxidized palm, oil feeding was greater than (Group II) fresh palm oil-fed while greater than (Group 1) control (Table =2)

Effects of reused palm oil on Triglyceride of the albino mice (*Mus musculus Linnaeus 1758)*

Triglyceride levels in (Groups I) control groups were (167.83 ± 17.83 mg/dl), in (Group II) fresh palm oil-fed were (193.17 ± 18.67mg/dl) while in (Group III) thermally oxidized feeds (Groups III) were (315.33 ± 34.91mg/dl). There was altogether increment ($p<0.05$) in the triglyceride (Tg) substance in (Group III) thermally-oxidized palm oil as compared to (Group I)control as well as to the (Group II) fresh oil group (Table 3).

Effect of reused oxidized palm oil on HDL level of the albino mice (*Mus musculus Linnious* 1758)

HDL levels in (Group I) control were (67.17 ± 5.92mg/dl). In (Groups II) fresh palm oil feeding was (48.83 ± 4.47mg/dl), while in (Groups III) thermally oxidized feed was (23.67 ± 2.74 mg/dl). The (group II) and (group III) show decreased HDL levels when compared to the control groups (Table; 4)

Effect of reused oxidized palm oil in LDL level of the albino (*Mus musculus Linnious 1758)*

LDL levels in (Groups I) control groups were (34.6 ± 3.26mg/dl). In (Groups II) fresh palm oil feed was (38.81 ± 3.84mg/dl) and in (Groups III) thermally oxidized feed was (64.61 ± 6.28mg/dl). The mice (Group3) fed with oxidized palm oil and (Group II) fresh palm oil groups indicated critical increment ($p<0.05$) in LDL levels contrasted to (Group I) control(Table 5)

Effect of oxidized palm oil on histological examination of heart of the albino mice, (Mus musculus Linnious 1758).

The histopathology of heart of (Group III) feed thermally oxidized palm oils indicated few vacuolation and congested myocardial condition in papillary muscle (Figure; 1).

Table 1: Effects of reused Oxidative Palm Oil on body weight of the albino mice

S.No.	Group*	Primary body weight(g) (M±SEM)*	Final body weight(g) (M±SEM)*	Weight gain(g) (M±SEM)*
1	Control	244 ±4.95	268± 2.9	24 ± -2.86
2	Fresh palm oil	243± 16.05	296 ± 12.01	53.5 ± 4.04
3	Reused oxidized palm oil	233± 23.90	297± 13.30	64.11± 9.97

Table 2: Effects of re-used palm oil on Total Cholesterol of the albino mice (*M. musculus Linnaeus* 1758)

S. No.	Group*	N*	Total Cholesterol mg/dl (M±SEM)*
1	Control	8	131.65 ± 12.87
2	Fresh palm oil	8	252.33 ± 22.1
3	Thermally oxidized palm oil	8	311.23 ± 30.79
Control: feed with normal diet; N: number of M. musculus used for experiment; ; weight measure in gram (g); M±SEM: mean± standard error mean; oxidized palm oil: after heating of palm oil at 100 °C;			

Table 3: Effects of re-used oxidized palm oil in Triglyceride level of the albino mice (*Mus musculus* Linnious 1758)

S. No.	Group*	N*	Triglyceride mg/dl (M±SEM)*
1	Control	8	167.83 ± 17.83
2	Fresh palm oil	8	193.17 ± 18.67
3	Thermally oxide palm oil	8	315.33 ± 34.91
* Control: feed with normal diet; N: number of *M. musculus* used for experiment; weight measure in gram (g); M±SEM: mean ± standard error mean; oxidized palm oil: after heating of palm oil at 100 °C.			

Table 4 : Effect of reused oxidized palm oil in HDL level of the albino mice (*Mus musculus Linnious* 1758).

S. No.	Group*	N*	HDL* mg/dl (M±SEM)*
1	Control	8	67.17 ± 5.92
2	Fresh palm oil	8	48.83 ± 4.47
3	Thermally-oxidized palm oil	8	23.67 ± 2.74
Control: feed with normal diet; N: number of *M. musculus* used for experiment; weight measure in gram (g); HDL: high density lipoprotein; M±SEM: mean± standard error mean; oxidized palm oil: after heating of palm oil at 100 °C;			

Table 5: Effect of reused oxidized palm oil in LDL level of the albino mice (*Mus musculus Linnious 1758).*

S. No.	Group*	N*	LDL mg/dl * (M±SD)*
1	Control	8	34.6 ± 3.26
2	Fresh palm oil	8	38.81 ± 3.84
3	Thermally-oxidized palm oil	8	64.61 ± 6.28
Control: feed with normal diet; N: number of *M. musculus* used for experiment; weight measure in gram (g); LDL: Low density lipoprotein; M±SEM: mean± standard error mean; oxidized palm oil: after heating of palm oil at 100 °C;			

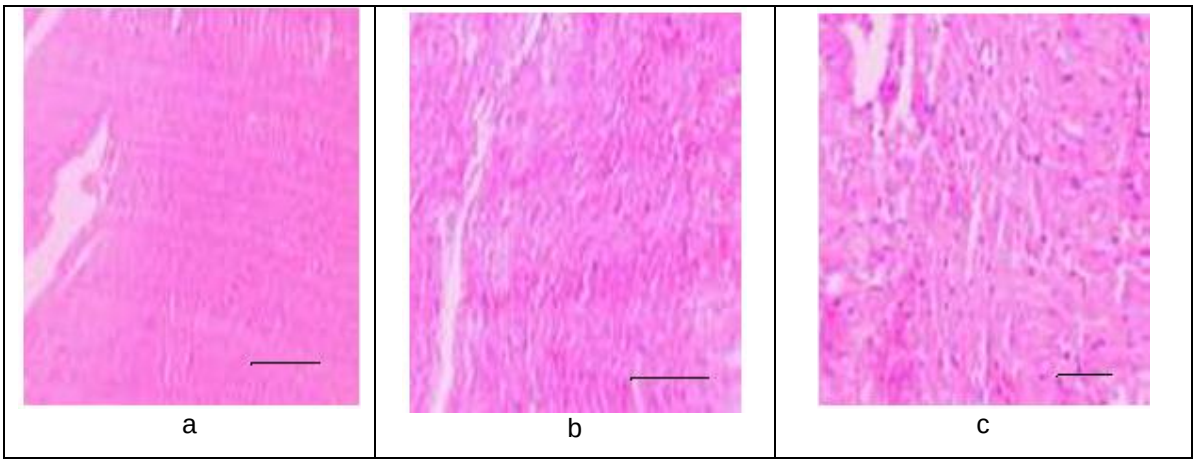

Figure1: the effects of oxidized lipid(palm oil) on histological alteration of heart of the albino mice; A (control): feed with normal diet; **B:** fresh palm oil, feeding group demonstrated congested myocardial condition, **C:** oxidized palm oil after heating of palm oil at 100 °C feeding group seen papillary muscle, few vacuolation: line on the photographs show 1 mm.

DISCUSSION

Nutrition plays a major role in preventing infectious diseases. Nutrition problems are common in the Asian country. Some developed countries have nutritional policies designed to tackle health problems thus the extant research was carried out to investigate that the oxidized palm oil has effects on the albino mice's serum lipid profile and histological heart alterations in the heart in the albino mice. The conclusion showed an increase in weight and cholesterol level ($p<0.05$), triglyceride, LDL contents in (group 3) thermally oxidized feeding albino mice. Oxidized lipid (palm oil) feeding group also demonstrated few vacuolation in heat muscle of albino mice, M. Musculus Linnaeus 1758.

Results revealed that, Group II (55.5 ± 4.04 g) and III (68.11± 9.97 g) mice gain significant ($p < 0.05$) body weight compare with control group which were more prominent in reused

oxidized palm oil. Karaji et al. (2006) inspected the impact of palm oil 12% on four-week aged mal rodents (148.3±10.7 g) for eight weak. The progressions of weight and sustenance admission were observed. These outcomes demonstrated that the mean estimation of rodent weight was expanded through vitality allow in count calories ($p< 0.05$). The triglyceride (Tg) substance was an increase in (Group III) thermally-oxidized palm, oil as compared to (Group I) control as well as to the (Group II) fresh oil group. Edem (2002) demonstrated that oxidized palm oil enlarged total cholesterol (T-C) in rodent the most noteworthy proliferation were apparent in oxidized palm oil (10 tpo) is an agreement with our present work.

Thermally oxidized feed was (23.67 ± 2.74 mg/dl). The (group 2) and (group 3) show decreased HDL levels when compared to the control groups and the mice (Group III) fed with oxidized palm oil and (Group II) fresh palm oil groups indicated critical increment ($p<0.05$) in LDL levels contrasted to (GroupI) control. Jane, et al. (2019) postulated that level of total cholesterol, triglyceride and LDL were increased approvingly with increase of thermo-oxidation duration of palm oil associated with the normal group, As the hours of frying increased, the cholesterol level grew irregularly, The levels of high-density lipid and cholesterol, were expanded essentially ($p< 0.05$), whereas the levels of low-density lipoprotein (LDL) and triglyceride were diminished however measurably not noteworthy. Taking everything into account, utilizing palm oil can help avert cardiovascular illness. In present research worked mice show an increase ($p<0.05$) in weight, (HDL), (LDL), and triglyceride. Karupaiah et al. (2016) also demonstrated that levels of little, thick low-density cholesterol the kind of cholesterol connected with coronary illness expanded due to eating palm oil. Whereas in the present study, the low-density lipoprotein (LDL) level is also increased so both the study is similar. According to Karaji et al. (2006) that regular eating of oxidized palm oil produces free radicals, oxidative stress, and abnormality in lipid profile. For that, we have analyzed the effect of recycled edible oils on body weight gain, the lipid profile of experimental mice Show similarity with our works.

It is clear from previous studies of Jaarin, (2016) that thermally oxidized palm, oil-eating expanded total cholesterol, and LDL level. In addition, they similarly described that oxidized low-density lipoprotein (LDL) is cytotoxic for causing ultra-structural changes in the rodent aorta. in the present research, we also find out that thermally oxide palm, oil caused a significant increase ($p<0.05$) in total cholesterol and bad LDL level and caused few vacuolation in hearth muscles and The histological study demonstrated the alteration in mass heart cells. Thus both research works are the same due to the same thermally oxidation method. Histological cardiac analyses of (Group III) feed with reused palm oils showed few vacuolations and congested myocardial state in the papillary muscle are supported with the statement of Dobarganes, and Marquez 2015). Prolonged use of frequently heated oil has been shown to raise blood pressure and total cholesterol induces artery dysfunction and vascular changes conditions that lead to atherosclerosis.

CONCLUSION

The study concludes that feeding of reused oxidized palm oil, persuades free radicals, oxidative stress, lipid profile abnormality, and cardiovascular disease. Further this plant extract can also be used scientifically for other fatal and chronic diseases. Hence this research recommends that the oil after being used possess the strong toxic property, which could be Avoided to used again. Thus further exploration of the toxicity is required.

Recommendation

It is suggested to reduce or avoid the use of reused oxidized palm oil. Additional inquiry is recommended to determine the effects that lead to the alteration in the histology of vital organs.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

B.A, D.NZ and T H designed A.A, A U H performleed the experiments I.K ,IU and M I wrote the manuscript. SA, IAS and AK performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. All authors read and approved the final version.

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