Jimma University

Institute of Health

Faculty of Medical Sciences

Department of Biomedical Sciences

The Effect of Palm Oil Fried Street Kokor on Lipid Profiles, Liver and Kidney Histopathology and Biomarkers of Swiss Albino Mice

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Jimma, Ethiopia

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Abstract

Background: Frying of foods, especially on streets with edible oils like palm oil alters the contents of oils and causes the formation of many harmful substances. Even though locally fried foods are consumed commonly in our society, their health effect is not studied well.

Objective: The aim of this study was to assess the effect of palm oil fried street kokor on lipid profiles, liver and kidney histopathology and biomarkers of Swiss Albino mice.

Methods: Thirty-two male and female Swiss Albino mice with age of 10-12 weeks old and body weight of 31.70 g to 47.10 g were divided randomly into four groups of eight members with equal male and female subgroups. The control group (Group-I) received only standard pellet and the experimental groups; Group-II, Group-III and Group-IV received 10%, 20% and 30% kokor of their daily food consumption respectively. The body weight of the mice was measured every week to assess the effect of the kokor on their body weight. At the end of the 6^{th} week, they were fasted for 12 hours and sacrificed by thoraco-abdominal incision after anesthetizing by diethyl ether. Blood was taken from each mouse by cardiac puncture and analyzed for lipid profiles, liver and kidney function tests. Liver and kidney tissues were taken from each mouse, fixed in 10% formalin solution for histopathological investigation. Data were entered to epi-data version 3.1 and analyzed by SPSS version 25.0.

Result: The body weight of the experimental groups was decreased significantly when compared with the control group ($P < 0.05$). The level of triglyceride (TG), low density lipoprotein (LDL) and total cholesterol (TC) of experimental groups male and female mice was increased significantly compared with the control groups ($P < 0.05$). There was significant decrement of high-density-lipoprotein (HDL) level of experimental groups male mice ($P < 0.05$) but not the females. The serum level of liver damage biomarkers (alanine transaminase (ALT) and aspartate transaminase (AST)) and kidney damage biomarkers (urea and creatinine) of experimental groups were increased significantly relative to the control groups ($P < 0.05$). Lymphocytic infiltrations, inflammations and fibrosis were detected on the liver and kidney of the experimental groups. Level of biochemical profiles (except HDL) and tissue damages increased as the dose of kokor increased.

Conclusions: Palm oil fried street kokor disturbed the serum lipid profiles. It also damages liver and kidney tissues. The damage was exacerbated as the dose of kokor increased.

Key words: Kidney damage, kokor, lipid profiles, liver damage, palm oil, mice, street food

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Abbreviations and Acronyms

4-AAP	4-Amminoantipyrine
ADP	Adenine diphosphate
ALT	Alanine transaminase / Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate transaminase /Aspartate aminotransferase
ATP	Adenine triphosphate
BMI	Body Mass Index
CE	Cholesterol Esterase
CHD	Coronary Heart Disease
CO	Cholesterol Oxidase
CVD	Cardiovascular Diseases
DAP	Dihydroxyacetone phosphate
GPO	Glycerol phosphate oxidase
GPx	Glutathione Peroxidase
HDL	High Density Lipoprotein
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
LMICs	Low-and-Middle- Income Countries
NAD^+	Nicotinamide Adenine Dinucleotide (Oxidized)
NADH	Nicotinamide Adenine Dinucleotide (Reduced)
NCDs	Non-Communicable Diseases
NIH	National Institutes of Health

N-RNCDs	Nutritional Related Non-Communicable Diseases
PV	Peroxide Value
ROS	Reactive Oxygen Species
SD	Standard Deviation
SOD	Superoxide Dismutase
SST	Serum Separator Tube
TC	Total cholesterol
TG	Triglyceride
TGD	Triglyceride Dimer
TGO	Triglyceride Oligomer
TPC	Total Polar Compounds
VLDL	Very Low Density Lipoprotein

1. Introduction

1.1. Background

Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors on streets (1). They are easily available and affordable for many parts of the population (2). Fried foods are among the street foods those are frequently consumed in many countries in different sanitary conditions and qualities (3–5).

Frying is a process in which a raw food is immersed in heated oil for a short time to obtain unique flavor, texture and attractive appearance (6).

Vegetable oils, mostly used for frying of foods, are good sources of unsaturated fatty acid and phytochemical compounds that are degraded easily during deep frying (7). At the time of frying of foods with oils, especially repeatedly, the physical and chemical appearances of the oils are changed. Some of the chemical changes occurred during frying of oils are hydrolysis, oxidation, polymerization (8,9).

Hydrolysis of oils and fats release free fatty acids in the blood. Increased level of free fatty acids in the blood in turn increases lipoproteins which causes the occurrence of atherosclerosis (10–12).

High temperatures of the frying processes produce high molecular cyclic fatty acid monomers, triglyceride dimer (TGD), triglyceride oligomer (TGO), aldehyde by-products, alcohol, hydrocarbons and acrolein through polymerization of fatty acids which have bad effects for the body cells (6,13).

Oxidation of oils during frying causes many biochemical alterations in the body. It alters the nature of enzymes, the status of antioxidants and causes the formation of lipid peroxidation and trans fatty acids (14,15).

Trans fatty acids are unsaturated fatty acids that contain at least one non- conjugated double bond in the trans configuration in which the presence of trans double bonds makes the fatty acyl chains to align straight resulting in fats that are more viscous (solid) at room temperature (16–18). They are formed when singlet oxygen reacts with double bonds in unsaturated fatty acids. At the same way, when free radicals generated during lipid oxidation, they can react reversibly to a double

bond to form a radical adduct. As soon as a double bond is reconstructed, trans configuration is formed because a trans double bond is more thermodynamically stable (16).

Since the primary lipid oxidation products such as peroxides and hydroperoxides are unstable, they react rapidly with each other to form secondary lipid oxidation products that affect the nutritional safety of oils (19).

The chemical reactions occurred during frying of foods with oil results the increment of free fatty acids, reactive oxygen species (ROS) and trans fatty acids. On the contrary, polyunsaturated fatty acids, phenolic compounds and antioxidants decrease (20).

A number of factors such as the type of oil and the addition guidelines, frying time and temperature, fryer design, oil turnover time, and frying media storage and handling once the deep-fat frying process has finished are known to influence the hydrolytic and oxidation kinetics (21,22). The degree of removal of important chemicals within the oils increases as the time and frequency of frying increases (23). When the oils are repeatedly exposed to extreme temperature, moisture and air, especially in deep-fat frying during food preparation, the cardiovascular protective benefits of vegetable oils become removed (19).

Foods with low content of polyunsaturated and high trans fatty acids are risks to develop cardiovascular diseases like coronary heart disease (CHD), ischemic stroke, hemorrhagic stroke (7,24,25). These foods also have the capability to induce organ failure and histopathology changes on different organs like heart, intestinal mucosa, liver and kidney (26,27).

The cellular damage of fried foods is highly dependent on the dose of fried foods eaten. Consumption of large amount of fried and snack foods disturb lipid metabolism and induce oxidative stress and eventually create toxic environment for cells (15,28).

1.2. Statement of the problem

Street foods are good alternatives of food and source of income for the society. In spite of these advantages, they are exposed for cross-contamination, have low quality, delivered by unsafe containers and sold in unclean environment (29,30).

Foods fried with different types of oils have altered contents and cause the formation of many harmful compounds (22). The formation of these harmful substances increase cholesterol level which in turn increases cardiovascular diseases (CVD) (31). Cardiovascular diseases, one of the non-communicable diseases are the leading causes of morbidity and mortality worldwide, account for approximately one-third of all deaths (32). Even though there are many risk factors for the occurrence of CVD, feeding habit is one of the risk factors (33).

Substances formed in chemical reactions during continuous frying of oils by high temperature form compounds with high molecular weight and polarity which have unfavorable effects to health (9). Total polar compounds (TPC), which are formed during the frying, processing and storage of fats and oils, have raised a great concern because of their adverse health effects. They cause lipid deposition, oxidative stress and cytotoxicity by deactivating genes of enzymes which are important for lipolysis (28).

The removal of antioxidants and the formation of free radicals have a synergetic effect on the damaging role of the process of frying on many aspects of health (23).

Chronic feeding of oxidized palm oil induces organ damages and dysfunctions. It causes decreases in renal plasma flow and glomerular filtration rate. It also causes the elevation of both systolic and diastolic blood pressures which cause glomerular injury (34). Fatty acid oxidation proved to cause insulin resistance, heart failure, CVD, cancer, etc.(15,35).

In United States, the incidence of heart failure among frequent consumers of fried foods is 4.1%. Frequent consumption of fried foods can also lead to overweight (obesity) which in turn results in elevated blood pressure, Type II diabetes in which all finally cause heart failure (36,37).

In India, street snack foods contain up to 69% saturated fats and 30% trans fats of total fatty acids (38). Trans-fatty acid chains may disorder the membrane phospholipids and influence the fluidity of the membranes. They have many bad consequences for cell activities and functions (39,40). They also increase low density lipoprotein (LDL), the plasma low density lipoprotein/high density

lipoprotein (LDL/HDL) ratio, and the total cholesterol ((TC)/HDL) ratio which have detrimental effects on cardiovascular health (41). High level of the above compounds in the blood causes atherosclerosis, a diseases characterized by accumulation of lipids and inflammatory cells within the intima of large arteries (42,43).

Even though Africa is not a victim of nutritional related non-communicable diseases (N-RNCD) so far, the burden of N-RNCD conditions may increase during the years to come if calorie availability increases. Though there are few data sources on actual prevalence of N-RNCDs in Africa, they (diabetes, cardiovascular disease and others) account for around one-third of all deaths (44).

The prevalence of overweight of under five children and reproductive age women in Ethiopia is 1.8% and 6% respectively (44). Hypertensive heart diseases and ischemic heart diseases account for 13.6% and 9.6% respectively from all cardiovascular patients in the country (45).

Since our country is a home of many diversified cultures, the food and feeding styles are also different. But the effects of these different foods and feeding styles on the health of the citizens are not well studied. This study tried to investigate the effect of palm oil fried street kokor on the lipid profiles, liver and kidney histopathology and biomarkers of Swiss Albino mice.

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1.3. Significance of the study

Among many factors affecting health, nutrition can be mentioned as a main determinant. Oils, important constituents of our day to day foods are thought to cause chronic non-communicable diseases. Especially, when they are heated repeatedly and fried openly on streets many health deleterious substances are formed. This study attempted to identify the effect of palm oil fried kokor on lipid profiles as well as liver and kidney functions and histopathology. So that the study delivers a right insight about the effect of palm oil fried street kokor on health. As a result, the study result may help health policy makers of the country as one input to propose appropriate nutrition related health policies. Moreover, the community can use the findings of the study to adjust its feeding style towards these foods. In addition, researchers can use the result of this study as a baseline for further evaluations and investigations related to this study.

2. Literature review

The habit of food consumption is highly related with the welfare of human being (46). Fast foods and fried foods are used frequently since they are simple and fast for preparation and uncomfortable to the breeding of microorganisms. But consumption of them is associated with cardiovascular diseases (2,47).

2.1. Overview of palm oil

Palm oil, among vegetable oils that obtained from the mesocarp of the fruits of the tropical plant palm tree (*Elaeis guineensis)*, is one of the oils used for frying of foods (48) and contains 50% saturated fatty acids, 39% mono-unsaturated fatty acids and 11% polyunsaturated fatty acids (49). It represents 30% of the world's vegetable oil production and its consumption has increased rapidly in the past several decades because it is the cheapest oil with greater oxidative stability. The two South- east Asia countries, Malaysia and Indonesia account for between 80-85% of the world's production of palm oil (50–52). In 1995, Ethiopia imported 7000 metric ton palm oil and reached to 395,000 metric ton in 2018 (53).

Palm oil and palm olein have been increasingly used in frying due to their techno-economic advantages over other oils/fats. It is estimated that several million tons of palm oil/palm olein, in their natural forms, in blends with other oils or after specific processing, are used annually for domestic and commercial frying services (54).

Oils are used largely to prepare fast foods and fried foods that are high in energy and fats content which leads to lifestyle diseases like diabetes, hypertension, cardiovascular diseases metabolic syndromes and soon (17).

Frying of different foods in oils has different effects on the content of the foods. A study done in Portugal and Spain to asses the effect of frying potatoes with different types of oils, pointed out alteration of the content of the oils in the potatoes. The study explained that after frying, the level of many hazardeous compounds like acrylamide, volatile compounds and TPC increase. It also indicated the frequency and duration of frying determine the degree of alteration (20).

Numerous studies describe that when palm oil is repeatedly exposed to extreme temperatures, moisture and air during frying cardiovascular protective benefits of vegetable oils deteriorate (55).

2.2. Effect of palm oil fried foods on body weight and lipid profiles

Serum lipid profiles are measured for cardiovascular risk prediction and has four basic parameters; triglyceride (TG), LDL, HDL and TC (56). TG is composed of a glycerol molecule bound to three fatty acids and are digested by pancreatic lipase. During hydrolysis it releases free fatty acids to the circulation which serve as a source of energy and deposited in different cells when they are excess (57). LDL, also known as beta lipoprotein, carries cholesterol from liver throughout the body. HDL, commonly known as the "good" cholesterol, is a heterogeneous collection of lipoprotein particles. It is used to the transportation of cholesterol from cells, promotion of nitric oxide production, prevention of endothelial apoptosis, suppression of proinflammatory molecules so that important for the prevention of atherosclerosis (58–60).

High level of cholesterol in the blood causes the formation of plaques within the vessels and results in atherosclerosis (43).

Studies are proving feeding foods that are cooked by palm oil, especially repeatedly fried, results in the perturbation of serum lipid profiles (41,51,61). During the process of frying foods by oils, excessive free fatty acids, trans fatty acids, peroxides, polar compounds and other harmful substances are formed which can cause the disturbance of biochemical profiles and affect the normal component of the body composition (62–64).

An experimental trial conducted in Brazil indicated that the study participants who ingested previously boiled crude palm oil showed an overall decrease in all lipid fractions, with a significant reduction in concentrations of very low density lipoprotein (VLDL) and TG and the concentration of HDL remains similar. The study also proved that boiled crude palm oil has no significant effect on the body mass index (BMI) of the participants (65). A similar finding was got from an investigation conducted in Malaysia to evaluate the effect of heated oils on lipid profiles and BMI of the rabbits. The animals were fed heated palm oil and corn oil and their BMI was decreased (51).

On the contrary, a double blinded randomized trial study done in Denmark to determine the effects of olive oil and palm olein on plasma cholesterol evaluated that palm olein has a greater effect on the level of TC and LDL to increase compared with olive oil. But it has a lower effect to increase the BMI of the participants than the olive oil (66).

Similarly, a laboratory based experimental trial held in Bangladesh determined the effect of long time heated palm oil on hematological, biochemical and lipid profiles and organ tissues of rabbits. In the study, it has been appreciated that the long time the oil heated, the more the rabbits weight increase. Biochemical profiles of serum lipid level such as TC, LDL and TG in blood of heated palm oil diet fed rabbits increased significantly. HDL also showed a little increment (67,68).

In Ethiopia, several types of edible oils, including palm oil, are used for cooking purposes. A study conducted at Bahirdar, Ethiopia to compare the effect of niger seed oil, palm oil and sunflower oil on lipid profiles showed that the effect of palm oil on the levels of TG, LDL, HDL and TC was higher than sunflower and niger oils. Palm oil also had higher effect to increase the weight of the study rats (69).

2.3. Effect of palm oil fried foods on liver

When palm oil is used to fry foods repeatedly, harmful substances are formed and body tissues like liver and kidney are damaged which results the release of their contents (2). Alanine aminotransferase (ALT) is found mainly in liver, and much less in kidneys, heart or skeletal muscle. It is a more specific indicator of liver inflammation than aspartate aminotransferase (AST), as it may be increased in other organ diseases (70,71). Several studies have been conducted to understand the effects of palm oil fried foods on body organs and tissues, and significant findings have been found.

An experimental study on Winstar rats in Hyderabad, India, conducted to evaluate the health effects of consumption of repeatedly heated vegetable oils showed that they cause the increment of BMI and the enlargement of liver. The study also indicated repeatedly heated vegetable oils have more peroxide value, polyps of colon, increased level of serum antioxidant enzymes(superoxide dismutase (SOD), glutathione peroxidase (GPx)), and reduced level of glucose (15).

Long time heated palm oil alters organ tissues. It is appreciated that the longer time the oil heated, the more the rabbits weight increase. The serum level of ALT and AST are also increased (68).

Another study on Swiss albino rats in China was conducted to investigate the effects of polar compounds generated from deep-frying process of palm oil on lipid metabolism. In this investigation, the concentrations of plasma TC and TG was increased in all high-fat diet-fed groups

compared to basal diet-fed group rats fed with deep fried oils. The serum level of ALT and AST also increased dramatically in high-fat diet-fed rats compared to those in the low diet-fed group. The livers of high-fat diet-fed rats had higher contents of fatty acids with less unsaturated fatty acids compared to the liver of low-fat diet fed rats (72).

But other studies reported different somewhat from the above findings. For instance, a study done in Nigeria to evaluate the effect of thermally oxidized palm oil diets at biochemical indices in rats indicated that there is a significant decrease in the plasma and liver total protein, TG, HDL of the test groups and an increase in the activities of ALT, AST, total bilirubin, TC, LDL of the test groups compared to the control groups (61).

Investigations express that oils fried repeatedly lose their capability to scavenge free radicals. Palm oil loses its antioxidant ability as its duration and frequency of frying increases. Antioxidants such as α-Tocopherol and γ-Tocotrienol with in palm oil are degraded faster than other oils due to more oxidation of fatty acids (23).

2.4. Effect of palm oil fried foods on kidney

Urea and creatinine are the most frequently ordered tests to examine the kidney function. Urea is produced by the breakdown of proteins and is excreted in urine whereas creatinine is a non-protein nitrogenous compound that is produced by the breakdown of creatine in muscle and excreted by glomerular filtration at constant rate. Creatinine is more reliable indicator of renal function than urea since it is less influenced by other factors such as diet and hydration. But the serum level of urea is affected by diet, liver function, hydration, circadian rhythm and intestinal absorption. Both increase when there is damage of kidney (57,73).

M. H. Morshed and his colleagues studied the effects of long time heated palm oil on the kidney of rabbits. They proved that the heated palm oil induces damage on kidney. Feeding of heated palm oil increased the serum uric acid and creatinine level. There were also lesions on the kidneys and other organs of the rabbits those have been fed heated palm oil (68).

Studies conducted on animals indicate that there is a mild cystic dilatation in the tubules in corticomedullary junction and appearance alterations of the kidney of palm oil fed rats. Palm stearin causes a significant change in the serum uric acid and creatinine level of the control and the experimental groups (26,74).

A study in Nigeria to investigate the effect of thermally oxidized palm oil on Wistar rats showed that thermally oxidized palm oil induces vacuolation and degeneration of renal corpuscles, patchy/diffuse denudation of renal tubular cells with loss of brush border and accumulation of homogenous substances with eosinophilic debris. It also expressed thermally oxidized palm oil increases significantly the body weight (27).

Different studies in different set ups and areas have different findings regarding to the effect of oil fried foods on the biochemical, hematological and histopathological changes. Some of them proved feeding fried foods increases serum lipoprotein level and the others found that feeding fried foods have no effect and some other foods fried with oils decrease serum lipoprotein level. There isn’t a consistent agreement about the adverse impact of fried foods on health.

2.5. The effect of frying Kokor with palm oil and Conceptual framework

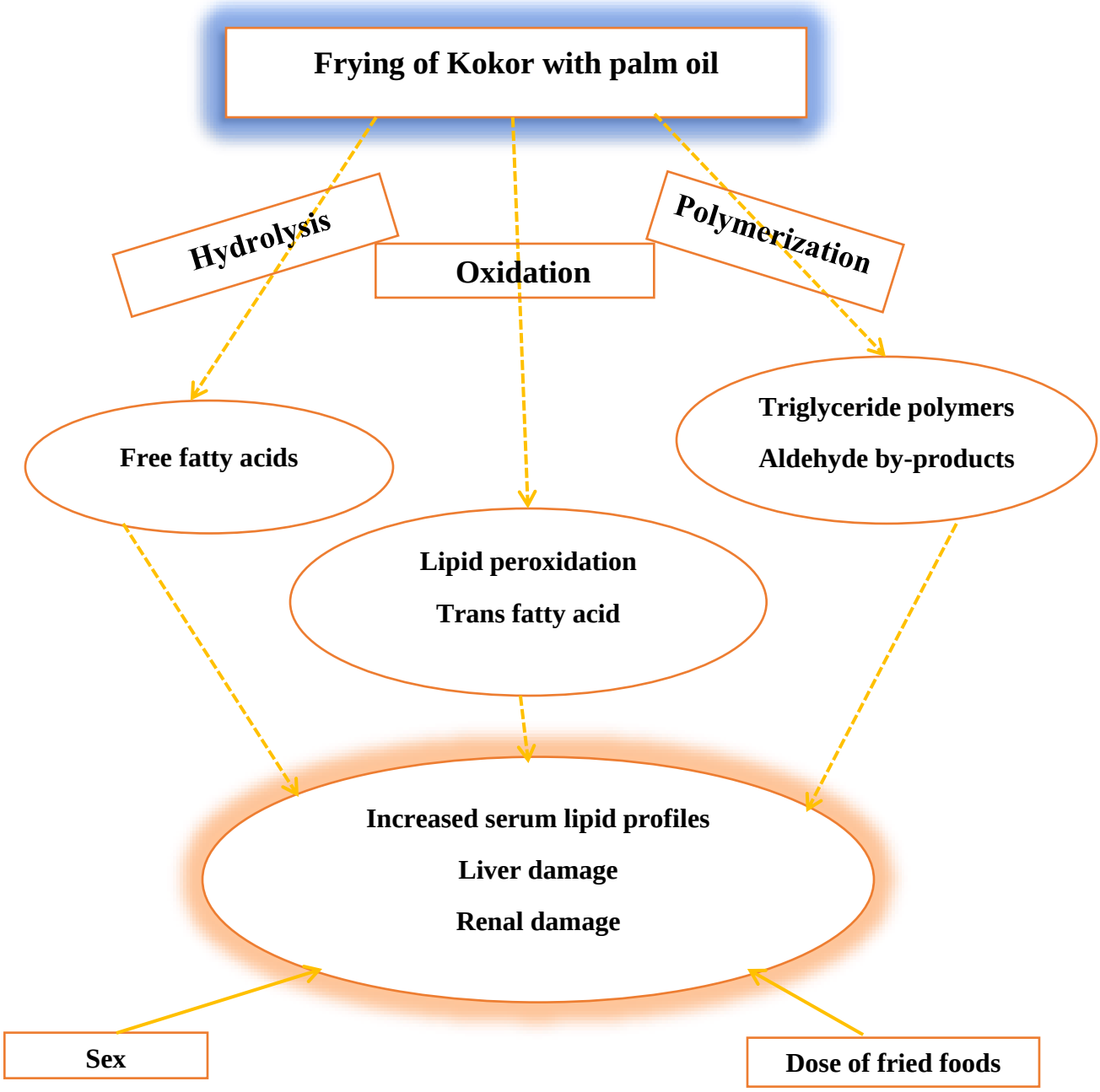

Figure 1: Conceptual framework and effect of frying adapted by reviewing different literatures (9–13,15)

2.6. Hypothesis of the study

Null hypothesis: Palm oil fried street kokor has not a significant effect on the level of lipid profiles, liver and kidney histopathology and biomarkers of Swiss Albino mice.

Alternate hypothesis: Palm oil fried street kokor has a significant effect on the level of lipid profiles, liver and kidney histopathology and biomarkers of Swiss Albino mice.

3. Objectives

3.1. General objective

The main objective of this study was to assess the effect of palm oil fried street kokor on lipid profiles, liver and kidney histopathology and biomarkers of Swiss Albino mice.

3.2. Specific objectives

1. To evaluate the effect of palm oil fried street kokor on Body weight of Swiss Albino mice
2. To analyze the effect of palm oil fried street kokor on lipid profiles (TG, LDL, HDL, TC) of Swiss Albino mice
3. To determine the effect of palm oil fried street kokor on liver functions (ALT and AST) of Swiss Albino mice
4. To examine the effect of palm oil fried street kokor on liver tissue of Swiss Albino mice
5. To determine the effect of palm oil fried street kokor on kidney functions (Urea and Creatinine) of Swiss Albino mice
6. To investigate the effect of palm oil fried street kokor on kidney tissue of Swiss Albino mice

4. Methods and Materials

4.1. Study area and period

The study was conducted in Veterinary Medicine Postgraduate Laboratory, Veterinary and Agriculture College, Jimma University, Jimma, Ethiopia from April 15 to May 28/2019.

4.2. Study design

Laboratory based randomized control experimental trial was conducted.

4.3. Sample size determination

The sample size was determined on the basis of resources. In resource equation method the value of E (degree freedom) is calculated based on decided sample size. The value "E" should lie within 10 to 20 for optimum sample size. If a value of E is less than 10 then more animal should be included and if it is more than 20 then sample size should be decreased.

E = Total number of animals - Total number of groups

E = 32-8= 24. Even though E is greater than 20, because the role of resource equation method is to guide as a rule of thumb, the sample size was determined to be thirty two (75,76).

4.4. Experimental animals and their grouping

Thirty-two (16 males and 16 females) Swiss Albino mice, with the age ranged from 10-12 weeks, were obtained from Jimma University Tropical and Infectious Disease Research Center, Sokoru, Ethiopia. They were brought to Jimma University Veterinary Medicine Postgraduate Laboratory and free to have access to food and distilled water ad libithum in accordance with the National Institutes of Health (NIH) Guidelines for Care and Use of Laboratory Animals (77). The mice were allowed to share the same environmental condition and exposed 12-hours light/dark cycle and acclimatized for one week before the beginning of the experiment. Then, they were divided randomly into 4 groups, having eight members each. Each group was also again categorized randomly into two subgroups as male and female subclasses in different stainless steel and plastic cages which were cleaned daily.

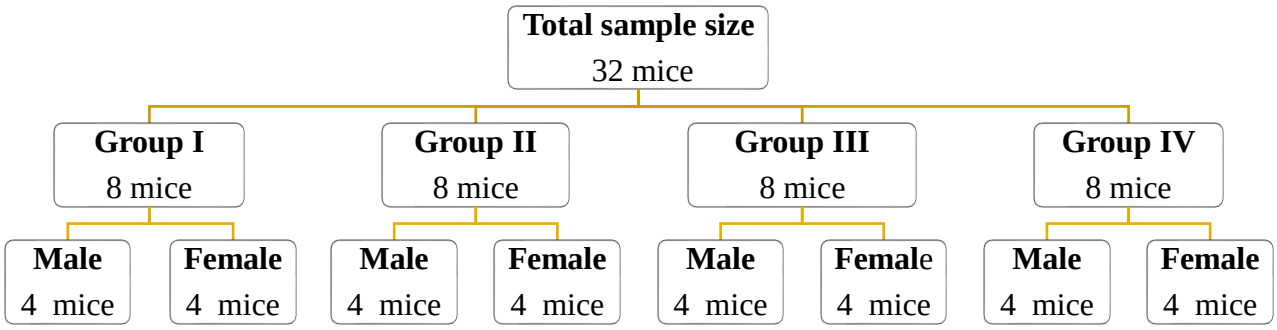

Figure 2: Random allocation of Swiss Albino mice into groups for the experiment

4.5. Experimental diet preparation and dosing procedures

Palm oil (3 L), wheat flour (5 kg) and commercial yeast were purchased from local markets in Jimma town, Ethiopia. The kokor was prepared based on local procedures in collaboration with street food vendors at four different streets of Jimma town at 5:00-6:00 pm for five days as follows.

1. The wheat flour was stirred with distilled water at a bowl and one teaspoon commercial yeast was added to fasten baking.
2. Then, it was stirred and kneaded until it became smooth and elastic.
3. After 8 hours, the dough was kneaded again and small ball shaped kokors (50-60 g) were made and fried by palm oil in a frying pan fueled by charcoal. The oil was not changed or no new oil was added until twenty-five (25) kokors were fried by classifying them in five batches for five minutes each batch.
4. Then, the kokors were shredded into small pieces, dried by sun for three days and ground by mortar and pestle until it became powder.
5. The powder was stored in a clean and dry glass container until administration.

Group-I (the control group) received only standard chow /pellet/ ad libithum and the experimental groups; Group-II, Group-III, Group-IV received 10%, 20% and 30% kokor of their daily food consumption respectively according to their body weight (55,78). This is due to fried foods constitute 7% and 21% of the total food consumed per day in Spain and India respectively(15,79). Mice eat 10% of their body weight per day(80). For the sake of administering the kokor easily, the daily total dose of each mice was divided into two equal doses and dissolved by distilled water in a concentration of 300 mg/ml and each half dose was given in 12 hours intervals through 24 gauge

oral gavage needle for six weeks(15,81). The dose of the experimental diet was calculated by the following formula.

$$D = \frac{BWx10}{100} x\ K$$

Key

D = Dose of kokor given to each mouse per day (mg)

BW = Body weight of each mouse (g)

$\frac{10}{100}$ = Daily food consumption of mice relative to their body weight

K = Proportion of kokor given to each mouse in a specific group (%)

4.6. Equipments

In this study, mortar and pestle, stainless steel and plastic cage, balance(Mettler Toledo MS104S, Switzerland), beaker (100ml, 400ml), syringe(3ml and 5ml), oral gavage needle(24 G), aluminum foil, surgical blade, metallic spatula, camera, test tubes, ice box (-20^{o}C), scissors, serum vials, centrifuge, automated chemistry analyzer (ABX Pentra 400, China), tissue processing machine (Leica TP1020, Germany), tissue embedding machine (Sakura Tissue Tek), wax dispenser, automated microtome (Leica RM2245, Germany), forceps, oven, tissue cassettes ,tissue slides, staining racks and right microscope were used.

4.7. Chemicals and reagents

- Lipid profiles reagents
- Liver function test reagents
- Renal function test reagents
- Tissue histopathology reagents
    - Ethanol (40%, 70%, 80%, 95%, 100%), diethyl ether, formalin (10%), paraffin wax, water, xylene, H&E stain.

4.8. Data collection procedures

The weight of the mice was measured every week to evaluate the effect of kokor on body weight using a checklist (Annex V). At the end of the 6th week, they were fasted for 12 hours and sacrificed by thoraco-abdominal incision after asphyxiating by diethyl ether (82). Five milliliter of blood was taken from each mouse through cardiac puncture and collected by a plain tube containing serum separator tube (SST) gel. The serum was separated through centrifugation with speed of 3000 revolutions per minute at room temperature for 10 minutes and put in ice-box and finally analyzed for lipid profiles (TG, LDL, HDL, TC) (Annex I), liver function tests (ALT and AST) (Annex II)) and kidney function tests (urea and creatinine) (Annex III). The liver and kidney tissues were taken from each mouse, washed with 0.9% normal saline and fixed in 10% formalin solution for histopathological evaluation (Annex IV).

4.9. Study variables

4.9.1. Dependent variables

- Serum lipid profiles (TG, LDL, HDL, TC) level
- Body weight
- Serum AST and ALT level
- Liver histopathology
- Serum urea and creatinine level
- Kidney histopathology

4.9.2. Independent variables

- Sex
- Dose of kokor

4.10. Data quality assurance

Prior to analysis, pre-analytical, analytical, and post-analytical precautions were considered and the equipments were calibrated through known standards. All manipulations and procedures conducted on the samples were done by trained professionals. Serum lipid profile levels (TG, HD, LDL and TC), liver function tests (AST, ALT) and renal function tests (urea, creatinine) were analyzed by ABX Pentra 400 clinical chemistry auto analyzer (HORIBA ABX SAS, China) as per

the manufacturer's instructions. Liver and kidney tissues were also analyzed by histologist and pathologist.

4.11. Data analysis procedures

Data were entered to epi-data software version 3.1 and then exported to SPSS version 25.0 software for analysis after it was checked and cleaned. Results were presented by tables and figures and expressed as mean ± SD. Statistical data analysis was done using paired t-test and one-way ANOVA post hoc multiple comparisons (Bonferroni) (Annex VI) and $P \leq 0.05$ was considered as statistically significant.

4.12. Operational definitions

- Kokor: small circular bread prepared from overnight baked wheat flour by frying with oil.
- Control group/mice: the group/mice which was/were given only pellet ad libithum.
- Experimental groups/mice: groups/mice which were given different doses of kokor.
- Liver function tests: are blood measurements which provide information about the existence, type and extent of liver damage.
- Kidney function tests: are blood measurements which provide information about the existence, type and extent of kidney damage

4.13. Ethical considerations

The research was conducted after getting ethical approval from Jimma University ethical reviewing committee and support letter for College of Agriculture and Veterinary medicine was written. All procedures on mice were in accordance with National Institutes of Health (NIH) Guidelines for Care and Use of Laboratory Animals.

4.14. Dissemination plan

The research result report was submitted to Jimma University Institute of health and Graduate programs coordinating office. It will also be disseminated by presenting at workshops and by publishing on reputable journal.

5. Results

Thirty-two Swiss Albino mice (16 males and 16 females) with the age ranged from 10-12 weeks were evaluated in the study. Their initial weight was ranged 31.70 g to 47.10 g with mean of 37.40 ± 2.00 g. Main findings of the study were analyzed below.

5.1. Body weight of the mice

Paired t-test was used to compare the initial and final body weight change of the mice. The final body weight of Group-I male mice (42.98±4.21 g) was increased significantly when compared with the initial body weight of the group (39.88±4.50 g) ($P < 0.05$). Similarly, the final body weight of Group-II (37.49 ± 2.30 g) and Group-III (35.16 ± 2.08 g) male mice was increased slightly when compared with their initial body weight but wasn't significant ($P > 0.05$). But the final body weight of Group-IV male mice (38.20 ± 1.32 g) was decreased a little when compared to its initial body weight which was not also significant ($P > 0.05$). Concerning of the female mice, the final body weight of Group-I (41.38±1.92 g) and Group-III (35.60±1.40 g) female mice was increased slightly when compared with their initial body weight but wasn't significant ($P > 0.05$). However, the final body weight of Group-II (36.28±2.32 g) and Group-IV (37.40±1.00 g) female mice was decreased slightly when compared with their initial body weight but wasn't significant ($P > 0.05$).

Table 1: Mean weight difference of the final and initial body weight of the mice

Groups	Sex of mice	Initial body weight (g)	Final body weight (g)	Mean difference ± SD	P-value
Group I	Male	39.88±4.50	42.98±4.21	-3.10±0.501[a]	0.001
	Female	39.40±2.12	41.38±1.92	-1.98±3.34[b]	0.322
Group II	Male	36.71±1.58	38.70±1.66	-1.99±2.75[b]	0.243
	Female	36.95±2.01	36.28±2.32	0.68±2.25[b]	0.591
Group III	Male	35.38±3.74	34.73±2.76	0.65±5.05[b]	0.813
	Female	34.13±1.89	35.60±1.40	-1.46±1.37[b]	0.121
Group IV	Male	38.40±2.31	39.00±1.17	0.65±5.05[b]	1.174
	Female	38.27±0.70	37.40±1.00	0.87±0.90[b]	0.152

The results were expressed as mean ± SD. Values with different superscripts within the same column are statistically significant ($P < 0.05$)

The final body weight of Group-I (control group) male mice (42.98±4.21 g) was slightly greater than the final body weight of Group-II male mice (38.70±1.66 g) and the final weight of Group-IV male mice (39.00±1.17 g) in which both differences were insignificant ($P > 0.05$). But the final body weight of Group-III male mice (34.73±2.76 g) was decreased significantly when compared with the final body weight of Group-I male mice ($P < 0.05$). On the other hand, the final body weight of all the experimental groups female mice was significantly less than the final body weight of Group-I (control group) female mice (41.38±1.92 g) ($P < 0.05$). There was no significant difference among the final body weight of the experimental groups male and female mice ($P > 0.05$).

Table 2: Final body weight of the groups of male and female mice.

Groups	Final body weight (g)	
	Male	Female
Group-I	42.98±4.21^{a}	41.38±1.92^{a}
Group-II	38.70±1.66^{ab}	36.28±2.31^{b}
Group-III	34.73±2.76^{b}	35.60±1.40^{b}
Group-IV	39.00±1.17^{ab}	37.40±1.00^{b}

The results were expressed as mean ± SD. Values with different superscripts within the same column are statistically significant ($P < 0.05$).

5.2. Serum lipid profiles of the mice

The serum lipid profiles (TG, LDL, HDL and TC) of the study mice were analyzed below. According to this study, there was no significant difference of all levels of lipid profile values between male and female mice ($P > 0.05$).

Table 3: Serum lipid profiles of male and female mice

Sex of mice	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	TC (mg/dl)
Male	141.92 ± 11.93^{a}	26.58±9.54^{b}	40.38±7.24^{c}	105.09±8.01^{d}
Female	138.88 ± 13.14^{a}	27.90±6.97^{b}	38.55 ±4.71^{c}	104.64±8.28^{d}

The results were expressed as mean ± SD. Values with different superscripts within the same column are statistically significant ($P < 0.05$).

The serum lipid profiles of the groups of male and female mice are given at table 4.

Table 4: Serum lipid profiles of the groups of male and female mice

Variables	Sex of mice	Group-I	Group- II	Group-III	Group-IV
TG (mg/dl)	Male	128.54 ± 6.98^{a}	138.27 ± 8.89^{ab}	146.24 ± 7.49^{bc}	154.64 ± 5.47^{c}
	Female	123.45 ± 11.93^{a}	135.85 ± 8.25^{ab}	147.12 ± 7.66^{b}	149.11 ± 5.80^{b}
LDL (mg/dl)	Male	12.50 ± 4.62^{a}	26.60 ± 4.78^{b}	32.29 ± 2.08^{bc}	34.93 ± 2.39^{c}
	Female	21.60 ± 3.05^{a}	22.29 ± 5.66^{a}	32.78 ± 2.30^{b}	34.93 ± 2.00^{b}
HDL (mg/dl)	Male	50.96 ± 3.05^{a}	40.19 ± 4.96^{b}	36.06 ± 1.76^{b}	34.33 ± 1.48^{b}
	Female	42.60 ± 4.10^{a}	39.23 ± 7.20^{a}	37.31 ± 0.83^{a}	35.10 ± 1.19^{a}
TC (mg/dl)	Male	93.65 ± 5.46^{a}	104.42 ± 2.32^{b}	109.71 ± 2.23^{bc}	112.59 ± 1.45^{c}
	Female	96.92 ± 3.34^{a}	97.02 ± 3.37^{a}	111.73 ± 2.09^{b}	112.88 ± 1.20^{b}

The results were expressed as mean ± SD. Values with different superscripts within the same row are statistically significant ($P < 0.05$).

The serum TG level of Group-III (146.24±7.49 mg/dl) and Group-IV (154.64±5.47 mg/dl) male mice was increased significantly when compared with the serum TG level of Group-I male mice (128.54±6.98 mg/dl) and the serum TG level of Group-IV male mice was higher than the serum TG level of Group-II male mice (138.27±8.89 mg/dl) significantly ($P < 0.05$) but its increment when compared with Group-III male mice was insignificant ($P > 0.05$). Similarly, the serum TG level of Group-II male mice was higher than the serum TG level of Group-I male mice and less than the serum TG level of Group-III male mice but its difference with both groups of male mice was not significant ($P > 0.05$).

The serum TG level of Group-III (147.12±7.66 mg/dl) and Group-IV (149.11±5.80 mg/dl) female mice was increased significantly when compared with the serum TG level of Group-I female mice (123.45±11.93 mg/dl) ($P < 0.05$). Likewise, the serum TG level of Group-II female mice (135.85±8.25 mg/dl) was higher than the serum TG level of Group-I female mice but not significant ($P > 0.05$). There was no significant difference among the serum TG level of experimental groups female mice ($P > 0.05$).

The serum LDL level of the experimental groups male mice was increased significantly when compared with the serum LDL level of Group-I male mice (12.50±4.62 mg/dl) ($P < 0.05$). And the serum LDL level of Group-IV male mice was higher than the serum LDL level of Group-II male mice (26.60±4.78 mg/dl) significantly ($P < 0.05$) but its difference with the serum LDL level of Group-III male mice was insignificant ($P > 0.05$). Similarly, the serum LDL level of Group-II male

mice was higher than the serum LDL level of Group-I male mice and less than the serum LDL level of Group-III male mice but not significant ($P > 0.05$).

The serum LDL level of Group-III (32.78±2.30 mg/dl) and Group-IV (34.93±2.00 mg/dl) female mice was increased significantly when compared with the serum LDL level of Group-I female mice (21.60±3.05 mg/dl) ($P < 0.05$). In the same way, the serum LDL level of Group-III female mice was significantly higher than the serum LDL level of Group-II (22.29±5.66 mg/dl) female mice ($P < 0.05$). Similarly, the serum LDL level of Group-IV female was significantly higher than the serum LDL level of Group-II female mice ($P < 0.05$) but insignificantly increased when compared with the serum LDL level of Group-III female mice ($P > 0.05$).

The serum HDL level of the all experimental groups male mice was decreased significantly when compared with the serum HDL level of Group-I male mice (50.96±3.05 mg/dl) ($P < 0.05$). However, the serum HDL level of experimental groups female mice was decreased insignificantly when compared with the serum HDL level of Group-I female mice serum HDL level (42.60±4.10 mg/dl) ($P > 0.05$). The dose-wise decrement of the serum HDL level among experimental groups male and female mice was not significant ($P > 0.05$).

The serum TC level of the experimental groups male mice was increased significantly when compared with the serum TC level of Group-I male mice (93.65±5.46 mg/dl) ($P < 0.05$). In addition, the serum TC level of Group-IV male mice was higher than the serum TC level of Group-II male mice (104.42±2.32 mg/dl) significantly ($P < 0.05$) but its increment when compared with the serum TC level (109.71±2.23 mg/dl) of Group-III male mice was insignificant ($P > 0.05$). Similarly, the serum TC level of Group-II male mice was higher than the serum TC level of Group-I male mice and less than the serum TC level of Group-III male mice serum TC level but was not significant ($P > 0.05$).

The serum TC level of Group-III (111.73±2.09 mg/dl) and Group-IV (112.88±1.20 mg/dl) female mice was increased significantly when compared with the serum TC level of Group-I female mice (96.92±3.34 mg/dl) ($P < 0.05$). Similarly, the serum TC level of Group-III female mice was significantly higher than the serum TC level (97.02±3.37 mg/dl) of Group-II female mice and the serum TC level of Group-IV female mice was significantly higher than the serum TC level of Group-II female mice ($P < 0.05$) but insignificantly increased when compared with Group-III female mice ($P > 0.05$).

5.3. Liver damage biomarkers

5.3.1. Biochemical analysis

According to the study, there was no significant difference of serum ALT and AST levels between male and female mice.

Table 5: Serum level of ALT and AST enzymes of male and female mice

Sex of mice	ALT(U/L)	AST (U/L)
Male	37.85 ± 10.81^{a}	111.30 ± 26.30^{b}
Female	38.79 ± 11.25^{a}	114.48 ± 26.17^{b}

The results were expressed as mean ± SD. Values with different superscripts within the same column are statistically significant ($P < 0.05$).

There was significant increment of serum ALT and AST levels of experimental groups male and female mice when compared with the control groups male and female mice ($P < 0.05$).

Table 6: Serum level of ALT and AST enzymes of the groups of the male and female mice

Groups	ALT (U/L)		AST (U/L)	
	Male	**Female**	**Male**	**Female**
Group-I	23.60 ± 2.47^{a}	23.72 ± 2.18^{a}	78.54 ± 1.80^{a}	81.59 ± 3.10^{a}
Group-II	35.08 ± 2.70^{b}	35.63 ± 4.33^{b}	99.44 ± 6.59^{b}	103.90 ± 3.47^{b}
Group-III	40.92 ± 2.38^{b}	43.56 ± 4.35^{c}	121.48 ± 5.45^{c}	122.72 ± 7.56^{c}
Group-IV	51.82 ± 3.49^{c}	52.25 ± 1.38^{d}	145.75 ± 6.51^{d}	149.71 ± 2.75^{d}

The results were expressed mean± SD. Values with different superscripts within the same column are statistically significant ($P < 0.05$).

The serum ALT level of Group-III (40.92±2.38 u/l) male mice was insignificantly greater than the serum ALT level of Group-II male mice (35.08±2.70 u/l) ($P > 0.05$) and the serum ALT level of Group-IV male mice (51.82±3.49 u/l) was increased significantly when compared with the serum ALT level of Group-II and Group-III male mice ($P < 0.05$). Regarding to the ALT level of the female mice, the serum ALT level of Group-III female mice (43.56±4.35 u/l) was increased significantly when compared with the serum ALT level of Group-II female mice (35.63±4.33 u/l) and the serum ALT level of Group-IV female mice (52.25±1.38 u/l) was also increased significantly when compared with the serum ALT level of Group-III female mice ($P < 0.05$).

The serum AST level of Group-III male mice (121.48±5.45 u/l) was increased significantly when compared with the serum AST level of Group-II male mice (99.44±6.59 u/l) and the serum AST level of Group-IV male mice (145.75±6.51 u/l) was also increased significantly when compared with the serum AST level of Group-III male mice ($P < 0.05$). Similarly, The serum AST level of Group-III female mice (122.72±7.56 u/l) was increased significantly when compared with the serum AST level of Group-II female mice (103.90±3.47 u/l) and the serum AST level of Group-IV female mice(149.71±2.75 u/l) was also increased significantly when compared with the serum AST level of Group-III female mice ($P < 0.05$).

5.3.2. Histopathological findings

In this study, the liver tissues of the control group had normal appearances. On the other hand, there were histopathological changes of liver tissues at the experimental groups compared to the control group. There were local lymphocytic infiltrations and inflammations on the liver tissues of the Group-II mice liver. On the liver of Group-III mice, there were moderately diffused lymphocytic infiltrations and fat accumulations. Severely diffused lymphocytic infiltrations, fat accumulations and fibrosis were observed on the liver of Group-IV mice.

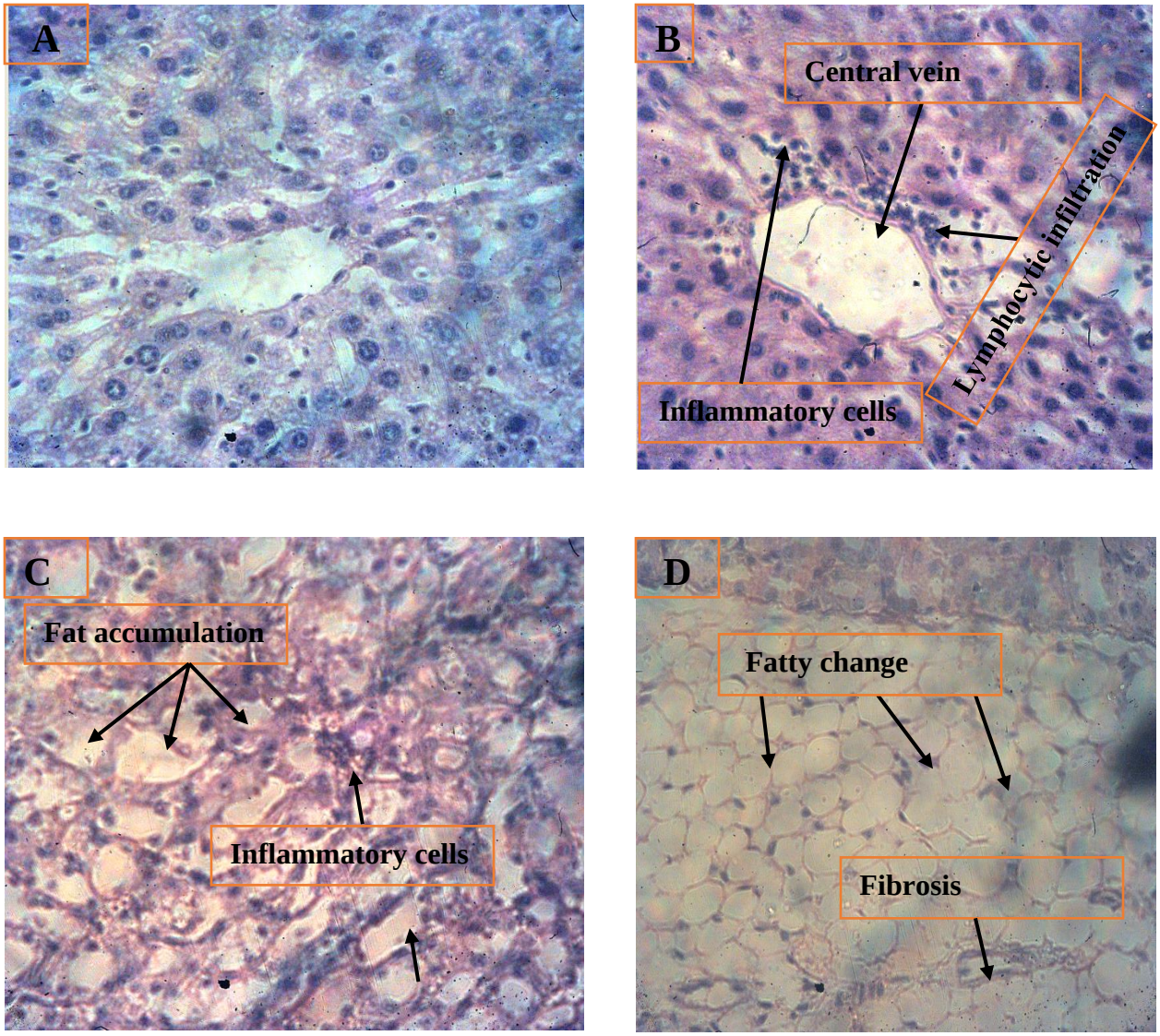

Figure 3: Photomicrograph of liver tissues of mice. **A** Group-I, **B** Group-II, **C** Group-III, **D** Group-IV (400X, H&E stain).

5.4. Kidney damage biomarkers

5.4.1. Biochemical analysis

In this study, there was no significant difference of serum urea and creatinine levels between male and female mice ($P > 0.05$).

Table 7: Serum urea and creatinine level of male and female mice

Sex of mice	Urea (mg/dl)	Creatinine (mg/dl)
Male	40.33 ± 0.66^{a}	0.74 ± 0.12^{b}
Female	40.31 ± 0.60^{a}	0.74 ± 0.10^{b}

The results were expressed as mean± SD. Values with different superscripts within the same column are statistically significant ($P < 0.05$).

The serum urea level of Group-III (40.77±0.78 mg/dl) and Group-IV male mice (40.86±0.23 mg/dl) was increased significantly when compared with Group-I (control group) male mice serum urea level (39.63±0.33 mg/dl) ($P < 0.05$). In the same way, the serum urea level of Group-II male mice (40.07±0.25 mg/dl) was also increased when compared with the serum urea level of Group-I male mice but not significant ($P > 0.05$). Equally, the serum urea level of Group-IV (40.83±0.12 mg/dl) female mice was increased significantly when compared with the serum urea level of Group-I (control group) female mice (39.59±0.22 mg/dl) ($P < 0.05$). Similarly, the serum urea level of Group-II (40.52±0.81 mg/dl) and Group-III (40.32±0.11 mg/dl) female mice was also increased when compared with the serum urea level of Group-I but not significant ($P > 0.05$). The increment of the serum urea level among experimental groups male and female mice wasn't significant ($P > 0.05$).

There was significant increment of serum creatinine levels of experimental groups male and female when compared with the control groups male and female mice respectively ($P < 0.05$). Among experimental groups, the serum creatinine level of Group-III (0.82±0.03 mg/dl) and Group-IV (0.84±0.02 mg/dl) male mice was increased significantly when compared with the serum creatinine level of Group-II male mice (0.75±0.02 mg/dl) ($P < 0.05$) but the difference between Group-III and Group-IV male was insignificant ($P > 0.05$). In similar way, The serum creatinine level of Group-III (0.81±0.01 mg/dl) and Group-IV (0.83±0.02 mg/dl) female mice was increased significantly when compared with the serum creatinine level of Group-II female mice (0.74±0.02 mg/dl) ($P < 0.05$) but the difference between Group-III and Group-IV female was insignificant ($P > 0.05$).

Table 8: Serum urea and creatinine level of the groups of the male and female mice

Groups	Urea (mg/dl)		Creatinine (mg/dl)	
	Male	**Female**	**Male**	**Female**
Group-I	39.63±0.33[a]	39.59±0.22[a]	0.56±0.02[a]	0.58±0.01[a]
Group-II	40.07±0.25[ab]	40.52±0.81[ab]	0.75±0.02[b]	0.74±0.02[b]
Group-III	40.77±0.78[b]	40.32±0.11[ab]	0.82±0.03[c]	0.81±0.01[c]
Group-IV	40.86±0.23[b]	40.83±0.12[b]	0.84±0.02[c]	0.83±0.02[c]

The results were expressed as mean ± SD. Values with different superscripts within the same column are statistically significant ($P < 0.05$).

5.4.2. Histopathological findings

In this study, the kidney tissues of the control group had normal appearances. On the other hand, there were histopathological changes of kidney tissues at the experimental groups compared to the control group. There were local lymphocytic infiltrations and inflammations on the kidney tissues of the Group-II mice kidney. On the kidneys of Group-III mice, there were moderately diffused lymphocytic infiltrations and local inflammations. Severely diffused lymphocytic infiltrations, and tubular necrosis were observed on the kidneys of Group-IV mice.

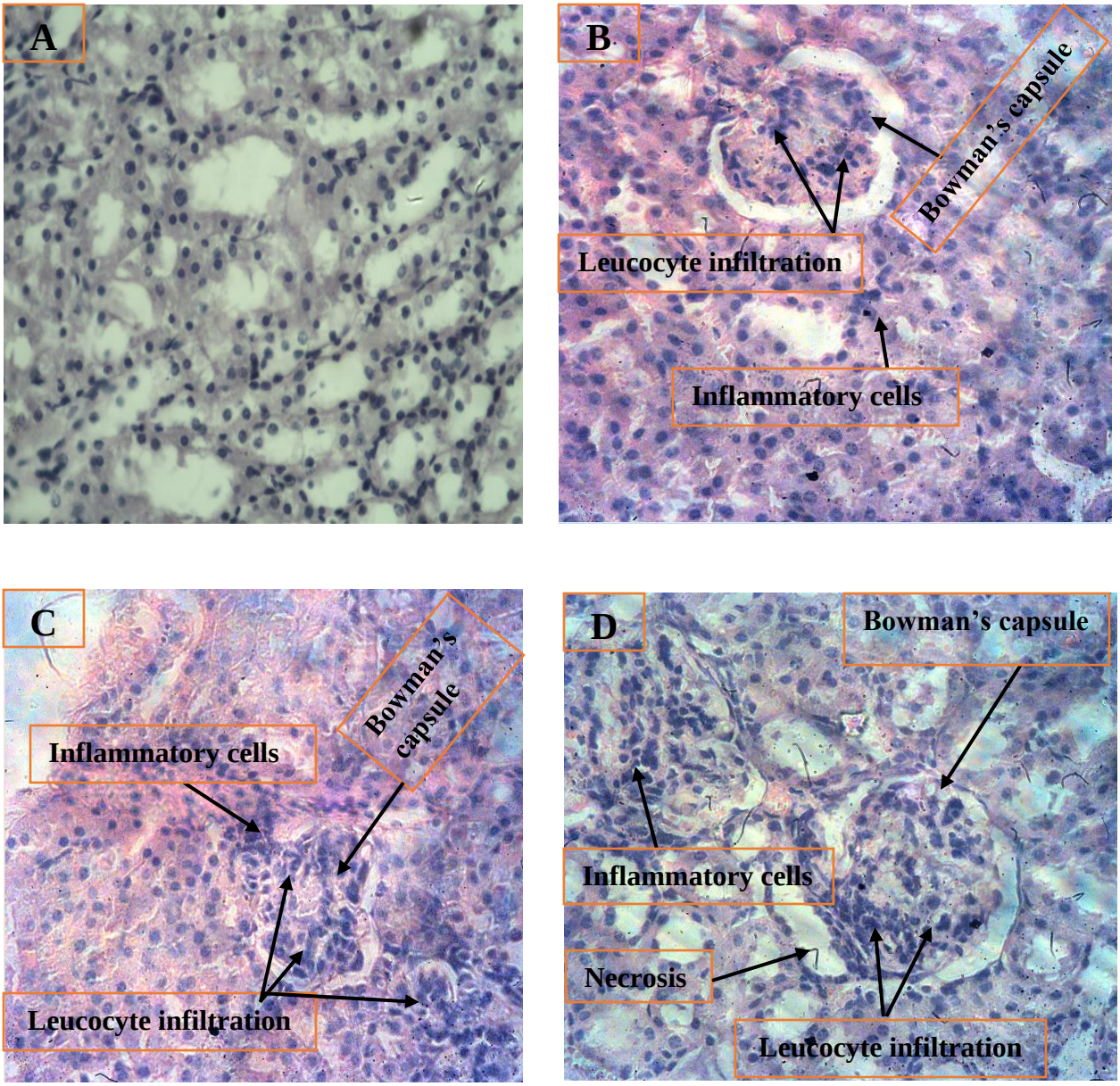

Figure 4: Photomicrograph of kidney tissues of mice. **A** Group-I, **B** Group-II, **C** Group-III, **D** Group-IV (400X, H&E stain)

6. Discussion

6.1. Body weight of the mice

Body weight is affected by several mechanisms including genetic, physiologic, and behavioral factors. Inappropriate dietary intake is among the contributing factors those affect body weight (83).

In this study, the final body weight of Group-III male mice (34.73±2.76 g) was decreased significantly when compared with the final body weight of Group-I male mice and the final body weight of all the experimental groups female mice was significantly less than the final body weight of Group-I (control group) female mice (41.38±1.92 g) ($P < 0.05$). This finding is consistent with the findings of A. Falade and his colleagues on rats in Nigeria and Idris and his colleagues found on rabbits in Malaysia in which both revealed that the weight and BMI of palm oil fed subjects is decreased (51,61) but didn't agree with Morshed and the colleagues' finding on rabbits in Bangladesh (68). The difference might be due to the physiological differences of the two experimental species (mice and rabbits). The body weight of the experimental groups male and female mice was also decreased as the dose of the kokor increased though it was not significant. This finding is concordat with the finding of Boniface M.N. and his colleagues on mice that describes as the dose of thermally oxidized oil increases, their body weight is decreased (27). The diminution of the body weight might be due to the cellular death of the mice in their body because of the effect of the oxidized substances formed during the frying of palm oil (15). The other proposed mechanism for the reduction of weight is due to the large polymers of lipid formed during frying of the palm oil which are less absorbable and also reduce the absorption of fat-soluble substances at gut that result in the decrease of their body weight (62).

6.2. Serum lipid profiles of the mice

The serum lipid profiles of the mice are given at table 4. In the present study, The serum TG level of Group-III (146.24±7.49 mg/dl) and Group-IV (154.64±5.47 mg/dl) male mice was increased significantly when compared with the serum TG level of Group-I male mice (128.54±6.98 mg/dl) and the serum TG level of Group-IV male mice was higher than the serum TG level of Group-II male mice (138.27±8.89 mg/dl) significantly ($P < 0.05$). Similarly, the serum TG level of Group-III (147.12±7.66 mg/dl) and Group-IV (149.11±5.80 mg/dl) female mice was increased significantly when compared with the serum TG level of Group-I female mice (123.45±11.93

mg/dl) ($P < 0.05$). The findings in this study are agreed to Morshed and his colleagues' findings that proved the repeatedly heated palm oil increases TG level of rabbits (68). The increment of TG in the serum of the experimental mice might be related with the increment of the free fatty acids formed during the frying process (84).

Fresh palm oil has the effect to lower serum LDL level but when oxidized it induces adverse effects on plasma lipid profiles, free fatty acids, phospholipids and cerebrosides (85). In the present study, the serum LDL level of the experimental groups male mice was increased significantly when compared with the serum LDL level of Group-I male mice (12.50±4.62 mg/dl) ($P < 0.05$). And the serum LDL level of Group-IV male mice was higher than the serum LDL level of Group-II male mice (26.60±4.78 mg/dl) significantly ($P < 0.05$). Similarly, the serum LDL level of Group-III (32.78±2.30 mg/dl) and Group-IV (34.93±2.00 mg/dl) female mice was increased significantly when compared with the serum LDL level of Group-I female mice (21.60±3.05 mg/dl) ($P < 0.05$). In the same way, the serum LDL level of Group-III female mice and Group-IV female mice was significantly higher than the serum LDL level of Group-II (22.29±5.66 mg/dl) female mice ($P < 0.05$). These findings are similar with other findings of previous studies by different scholars in different times and places that describe repeatedly heated palm oil increases serum LDL level (26,48,86). The increment of LDL in the serum of the mice could be related with high content of saturated fatty acids and trans fatty acids in the palm oil fried kokor (48,87–89).

Foods with higher energy and fat content but lower in vegetables and grains have reducing effect on the level of serum HDL (90). In this study, the serum HDL level of the all experimental groups male mice was decreased significantly when compared with the serum HDL level of Group-I male mice (50.96±3.05 mg/dl) ($P < 0.05$). However, the serum HDL level of experimental groups female mice was decreased insignificantly when compared with the serum HDL level of Group-I female mice serum HDL level (42.60±4.10 mg/dl) ($P > 0.05$). The serum HDL level was reduced among experimental groups male and female mice inversely as the dose of the kokor increased but insignificantly ($P > 0.05$). The result of this study supports the findings of the previous studies by Ayodeji and Ilyas with their colleagues that reported oxidized palm oil decreases serum HDL level (61,67). The reduction of the HDL in the serum of the mice could be attributed to the trans fatty acids formed during the frying process of the kokor with the palm oil (18,40,89,91–93). But other previously studied works indicate that small increment of HDL was observed from experimental

groups those fed oxidized palm oil which might be due to the low amount of trans fatty acids in these studies as the formation of trans fatty acids depend on different factors (67–69).

Palm oil has hypercholesteremic as its half content is saturated fatty acids when used in the oxidized state (85). In this study, the serum TC level of the experimental groups male mice was increased significantly when compared with the serum TC level of Group-I male mice (93.65±5.46 mg/dl) ($P < 0.05$). In addition, the serum TC level of Group-IV male mice was higher than the serum TC level of Group-II (104.42±2.32 mg/dl) male mice significantly ($P < 0.05$). Furthermore, the serum TC level of Group-III (111.73±2.09 mg/dl) and Group-IV (112.88±1.20 mg/dl) female mice was increased significantly when compared with the serum TC level of Group-I female mice (96.92±3.34 mg/dl) ($P < 0.05$). Similarly, the serum TC level of Group-III and Group-IV female mice was significantly higher than the serum TC level (97.02±3.37 mg/dl) of Group-II female mice ($P < 0.05$). The present finding is in agreement with previously done studies conducted on mice and other laboratory animals that describe palm oil increases serum cholesterol level (19,94). The increase in the level of TC in mice fed with palm oil fried kokor might be attributed to the increment of saturated fatty acids (69,88,95).

6.3. Liver damage biomarkers

When liver tissue is damaged, AST and ALT are released into the bloodstream and raise the serum enzymes level. So the amount of AST and ALT in the blood is directly associated with the amount of tissue damages (96). As described on table 6, the serum ALT level of the experimental groups male and female mice was increased significantly when compared with the control groups male and female mice respectively ($P < 0.05$). The serum ALT level increased significantly among experimental groups male mice and experimental groups female mice as the dose of the palm oil fried kokor increased ($P < 0.05$). Similarly, the serum AST level of the experimental groups male and female mice was significantly increased when compared with the control groups male and female mice respectively ($P < 0.05$). The serum AST level increased significantly among experimental groups male mice and experimental groups female mice as the dose of the palm oil fried kokor increased ($P < 0.05$). The findings of this study support the result of other previously done studies on rabbits and rats which indicate the serum level of ALT and AST is increased for subjects those have taken oxidized palm oil in different forms and doses (68,72,97). The elevation of the two enzymes in the serum is indicator of liver tissue damage (98).

Correspondingly, it was observed that the liver tissues of the experimental groups were changed when compared to the control group liver tissue. On the liver of the Group-II mice, there were only local lymphocytic infiltrations and inflammations. The liver tissue of Group-III had moderately diffused lymphocytic infiltrations and fat accumulations. But there were severely diffused lymphocytic infiltrations, fat accumulations and fibrosis on the liver of Group-IV mice. As observed from the result of the tissue analysis, the level of liver damage was increased as the dose of the palm oil fried street kokor increased. This indicates the more the fried kokor dose, the more liver damage. This finding supports the result of the study conducted by A. Bianchi and his colleagues that describes palm oil fried foods have degradative effect on liver (94). The damage of the liver tissue is due to the lipoperoxidation and toxic substances which disintegrates the hepatocytes and the accumulation of the fatty acids within them (99).

6.4. Kidney damage biomarkers

As described on table 7, the serum urea and creatinine level of the experimental groups male mice and experimental groups female mice was significantly increased when compared with the control groups ($P < 0.05$) male and female mice. The serum urea level of Group-III (40.77±0.78 mg/dl) and Group-IV male mice (40.86±0.23 mg/dl) was increased significantly when compared with Group-I (control group) male mice serum urea level (39.63±0.33mg/dl) ($P < 0.05$). Equally, the serum urea level of Group-IV (40.83±0.12 mg/dl) female mice was increased significantly when compared with the serum urea level of Group-I (control group) female mice (39.59±0.22 mg/dl) ($P < 0.05$). There was significant increment of serum creatinine levels of experimental groups of both male and female when compared with the control group male and female mice respectively ($P < 0.05$). Among experimental groups, the serum creatinine level of Group-III (0.82±0.03 mg/dl) and Group-IV (0.84±0.02 mg/dl) male mice was increased significantly when compared with the serum creatinine level of Group-II male mice (0.75±0.02 mg/dl) ($P < 0.05$). On the same way, the serum creatinine level of Group-III (0.81±0.01 mg/dl) and Group-IV (0.83±0.02 mg/dl) female mice was increased significantly when compared with the serum creatinine level of Group-II female mice (0.74±0.02 mg/dl) ($P < 0.05$). The findings of this study are similar with the results of the previously done studies on rats which emphasize the harmful effect of oxidized palm oil to kidney (26,55,74). The damage of the kidney of the mice could be due to lipid peroxidation and the free radicals formed during the frying process of the kokor with palm oil (19,34).

In addition, it was observed that the kidney tissues of the experimental groups were changed when compared to the control group kidney tissue which had normal appearances. On the kidney tissue of the Group-II mice, there were only local lymphocytic infiltrations and inflammations. The kidney tissue of Group-III had moderately diffused lymphocytic infiltrations and inflammations. But there were severely diffused lymphocytic infiltrations and tubular necrosis on the kidney of Group-IV mice. The result is consistent with other studies conducted previously to evaluate the effect of repeatedly heated palm oil on mice and rats kidney that causes tubular necrosis, degeneration of the renal corpuscle and accumulation of eosinophilic debris (27,74). The damage of the kidney tissues might be related with the toxic substances formed during the frying process of the kokor with palm oil (27,99). The severity of the damage is proportional with the amount of the kokor given for the mice which agrees with the study by H. Morshed as period of heating of palm oil increases the damage of the organs also increases (68).

7. Conclusions

In this study, palm oil fried street kokor had significant effect on the reduction of body weight. It was also shown that consuming palm oil fried street kokor increased the level of serum lipid profiles of male and female mice (TG, LDL, and TC) significantly and proportionally but decreased HDL level of male mice significantly and inversely to the dose of the kokor.

In addition, palm oil fried street kokor damaged the liver and kidney tissues of the mice. The harmful effect of the kokor was exacerbated as the dose of the fried kokor increased. The damaging effect of the food was approved through biochemical and histopathological evaluations. The liver enzymes of ALT and AST increased significantly in the serum of the e palm oil fried street kokor fed mice and lymphocytic infiltrations, fibrosis and fat accumulations also were observed on liver and kidney tissues which are the manifestation of liver and kidney damage respectively. Similarly, the serum level of urea and creatinine of the experimental groups were increased significantly when compared with the control groups.

Generally, this study proved that the palm oil fried kokor prepared and sold on streets disturbed lipid profiles and damaged liver and kidney of Swiss Albino rats.

8. Recommendation

I recommend the following suggestions as the investigator of this study.

- Comprehensive investigations of the components of palm oil fried street kokor should be done to understand all its health risks.
- Health education should be initiated to the community to create awareness about the health effect of palm oil fried street kokor.
- Further studies should be conducted to examine the effect of palm oil fried street kokor on other body organs and overall health aspects.

9. Limitations of the study

The study had the following limitations.

- It didn’t identify the compounds formed in the frying process.
- It couldn’t determine the temperature limit that causes the formation of harmful substances.
- The effect of kokor fried by other edible oils on liver and kidney was not evaluated in the study.
- The study also didn’t include palm oil fried foods other than kokor.

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Annexes

I. Serum lipid profiles determination

Measurement of serum TG, LDL, HDL and TC was done using fully automated ABX Pentra 400 clinical chemistry analyzer according to the reagent manufacturer's instruction in Jimma University specialized teaching hospital.

1. Serum TG determination

TGs in the samples are hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by ATP to glycerol-3-phosphate and ADP in a reaction catalyzed by glycerol kinase. Glycerol-3-phosphate is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2) by glycerophosphate oxidase (GPO). The H_2O_2 then reacts with 4-aminoantipyrine (4-AAP) in a reaction catalyzed by peroxidase to yield a red colored quinonimine dye. The intensity of the color produced is directly proportional to the concentration of TGs in the sample when read at 520nm.

$$TG + 3H_2O \xrightarrow{\text{Lipase}} \text{Glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol -3-P + ADP}$$

$$\text{Glycerol -3-P + } O_2 \xrightarrow{\text{GPO}} \text{DHAP + } H_2O_2$$

$$H_2O_2 + \text{4-AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine + } H_2O\ (100)$$

2. Serum LDL determination

A direct homogeneous enzymatic assay for the quantitative determination of LDL is used in a two reagent formats. The first reagent contains Good's buffer [pH 6.8; *N*-(2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyaniline, sodium salt], cholesterol esterase (CE), cholesterol oxidase (CO), catalase, polyanions, and amphoteric surfactants, which selectively protect LDL from enzyme reaction.
The non-LDL reacts with cholesterol esterase (CE) and cholesterol oxidase (CO), producing hydrogen peroxide (H2O2), which is consumed by catalase. The second reagent contains Good's buffer (pH 7.0), 4-aminoantipyrene, peroxidase, sodium azide, and deprotecting reagent.
The nonionic surfactants remove the protecting agent from LDL, enabling the specific reaction of CE and CO with LDL. The resulting hydrogen peroxide yields color with Trinder's reagent and 4-

aminoantipyrene in the presence of peroxidase. The blue color complex produced has an absorbance of 500 nm which is proportional to the concentration of LDL in the sample (100).

1st step:

HDL, VLDL, and chylomicrons $\xrightarrow{CE + CO}$ cholestenone + H_2O_2

$2H_2O_2 \xrightarrow{Catalase}$ $2H_2O_2 + O_2$

2nd step:

LDL $\xrightarrow{CE+ CO}$ cholestenone + H_2O_2

H_2O_2 + chromogen $\xrightarrow{Peroxidase}$ quinine dye (101)

3. Serum HDL determination

The HDL liquicolor test is a direct homogeneous enzymatic assay for the quantitative determination of HDL. The test combines two steps, the first step eliminating chylomicrons, VLDL and LDL by specific enzymatic degradation. The next step determines the remaining HDL by the well-known enzymatic reaction cascade cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase. In the final reaction hydrogen peroxide oxidizes a chromogen (N-(2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyaniline) under the catalytic action of peroxidase. The resulting color change is monitored at 500 nm and directly proportional to the HDL concentration in the sample. Reaction principles are as follows:

1st step

LDL, VLDL and chylomicrons $\xrightarrow{CHE +CO}$ cholestenone + H_2O_2

$2H_2O_2 \xrightarrow{Catalase}$ $2H_2O + O_2$

2nd step:

HDL $\xrightarrow{CHE + CO}$ cholestenone + H_2O_2

H_2O_2 + chromogen $\xrightarrow{Peroxidase}$ quinine pigment (100,102).

4. Serum TC determination

Cholesterol is measured after a series of coupled reactions. Cholesterol esters are converted to cholesterol and fatty acids by an enzyme cholesterol esterase. Then cholesterol is oxidized with oxygen by cholesterol oxidase in to cholest-4-en-3-one and hydrogen peroxide (H_2O_2). The H_2O_2

then reacts with 4-Amminoantipyrine (4-AAP) by peroxidase enzyme to yield a red colored quinonimine dye. The reaction sequence is as follows:

Cholesterol ester + H_2O —Cholesterol Esterase→ Cholesterol + Fatty acid

Cholesterol + O_2 —Cholesterol Oxidase→ Cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + 4-AAP —Peroxidase→ Quinoneimine + 4 H_2O

The intensity of the red color produced is directly proportional to the TC in the sample when read at 530 nm (100,103).

II. Liver function tests

1. ALT assay method

In the reaction, ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD^+. The system monitors the rate of change in absorbance at 340 nm over a fixed time interval. The rate of change in absorbance is directly proportional to the ALT activity in the sample. ALT measurements are used in the diagnosis and treatment of liver and heart disease. The method is linear up to 800 IU/L.

L-alanine + α-ketoglutarate ⟷ (ALT) ⟷ pyruvate + L-glutamate

Pyruvate + NADH + H^+ ⟷ (Lactate dehydrogenase) ⟷ lactate + NAD^+ (100,104)

2. AST assay method

AST is a cellular enzyme present in many tissues such as heart, skeletal muscles, kidney, brain, liver, pancreas or erythrocytes. It exists in two isoforms, cytoplasmic and mitochondrial. The cytoplasmic isoenzyme is released into the blood during the moderate cell damage. On the other hand, the activity of the mitochondrial isoenzyme in blood increases during the severe cell damage. The determination of AST activity in serum is used mainly to assess the liver damage. AST in biological system catalyzes the following reaction:

Aspartate + α-ketoglutarate ⟷ (AST) ⟷ oxaloacetate + glutamate

Oxaloacetate + NADH + H^+ ⟷ (Malate dehydrogenase) ⟷ malate + NAD^+ (100,105)

The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+ proportional to the activity of AST present in the sample. The method is linear up to 800 IU/L.

III. Kidney function tests

1. Serum creatinine

Serum creatinine is measured based on the production of glycine, formaldehyde and hydrogen peroxide from creatinine that is catalyzed by creatininase, creatinase, and sarcosine oxidase (SOD). Finally, quinoneimine chromogen is produced by peroxidase (POD) which catalyzes the reaction of hydrogen peroxide with 4-aminophenazone and HTIBa. Creatinine concentration in the reaction is directly proportional to the color intensity of the quinonimine chromogen formed.

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatininase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatinase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + O_2 + H_2O \xrightarrow{\text{SOD}} \text{Glycine} + HCHO + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{HTIB} \xrightarrow{\text{POD}} \text{quinonimine chromogen} + H_2O + HI$$

2. Urea

Ammonium and carbonate are formed from urea by urease followed by reaction of ammonium with 2-oxoglutarate to form L-glutamate by glutamate dehydrogenase (GLDH) and the coenzyme NADH

$$\text{Urea} + 2\,H_2O \xrightarrow{\text{Urease}} 2\,NH_4^+ + CO_3^{2-}$$

$$NH4+ + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The rate of decrease in the NADH concentration is measured by spectrophotometer at 700/340 nm and the absorbance is directly proportional to the urea concentration.

IV. Tissues histopathology

The liver and kidney tissues were taken from each mouse carefully after the mice had been sacrificed after 6 weeks feeding duration. The tissues were excised using sterile scissor and taken by blunt forceps to preserving tissue with neutral 10% of formalin for 12 hours. Then the tissues were taken out from the preservative and washed by ethanol with concentrations of 70%, 80%, 95% and 100% and xylene solution were used to remove ethanol from the tissue and replace this ethanol with fluid that is readily miscible with paraffin wax which enhances the tissue to embed easily with the wax to form tissue blocks. Then after, the tissue block was sectioned into 4-5µm thickness by precision knives (microtome) and that section was immersed in water bath at 45^{o}C and the unfolded section was taken and dried by putting in an oven at 56^{o}C for 20 minutes. Then this dried section was stained with hemato-xylineosin (H&E) stain after removal of paraffin wax by using ethanol with a descending concentration and examined by using light microscope under 400X magnification. Images were taken by using microscope camera and pictures were read & interpreted by histologist and pathologist (106).

V. Body weight follow up checklist

Table 9: Checklist for follow up of weight of mice per week

Group	Sex	No.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group-I	Male	1.						
		2.						
		3.						
		4.						
	Female	1.						
		2.						
		3.						
		4.						
Group-II	Male	1.						
		2.						

		3.						
		4.						
	Female	1.						
		2.						
		3.						
		4.						
Group-III	Male	1.						
		2.						
		3.						
		4.						
	Female	1.						
		2.						
		3.						
		4.						
Group-IV	Male	1.						
		2.						
		3.						
		4.						
	Female	1.						
		2.						
		3.						
		4.						

VI. Post hoc analysis table

Table 10: Multiple comparison of serum biochemical profiles of the study mice using one-way ANOVA post hoc(Bonferroni)

Dependent variable	(I) Group number	(J) Group number	Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.
			Male		Female	
Final body weight	Group IV	Group II	4.27500	.274	5.10000*	.008
		Group III	8.25000*	.006	5.77500*	.003
		Group IV	3.97500	.362	3.97500*	.043
	Group II	Group I	-4.27500	.274	-5.10000*	.008
		Group III	3.97500	.362	.67500	1.000
		Group IV	-.30000	1.000	-1.12500	1.000
	Group III	Group I	-8.25000*	.006	-5.77500*	.003
		Group II	-3.97500	.362	-.67500	1.000
		Group IV	-4.27500	.274	-1.80000	1.000
	Group I	Group I	-3.97500	.362	-3.97500*	.043
		Group II	.30000	1.000	1.12500	1.000
		Group III	4.27500	.274	1.80000	1.000
TG	Group I	Group II	-9.73451	.505	-12.38938	.402
		Group III	-17.69912*	.030	-23.67257*	.014
		Group IV	-26.10619*	.002	-25.66372*	.008
	Group II	Group I	9.73451	.505	12.38938	.402
		Group III	-7.96460	.896	-11.28319	.549
		Group IV	-16.37168*	.049	-13.27434	.311
	Group III	Group I	17.69912*	.030	23.67257*	.014
		Group II	7.96460	.896	11.28319	.549
		Group IV	-8.40708	.779	-1.99115	1.000
	Group IV	Group I	26.10619*	.002	25.66372*	.008
		Group II	16.37168*	.049	13.27434	.311
		Group III	8.40708	.779	1.99115	1.000
LDL	Group I	Group II	-14.09722*	.001	-.69444	1.000
		Group III	-19.79167*	.000	-11.18056*	.005
		Group IV	-22.43056*	.000	-13.33333*	.001
	Group II	Group I	14.09722*	.001	.69444	1.000
		Group III	-5.69444	.296	-10.48611*	.008
		Group IV	-8.33333*	.046	-12.63889*	.002
	Group III	Group I	19.79167*	.000	11.18056*	.005
		Group II	5.69444	.296	10.48611*	.008
		Group IV	-2.63889	1.000	-2.15278	1.000
	Group IV	Group I	22.43056*	.000	13.33333*	.001
		Group II	8.33333*	.046	12.63889*	.002
		Group III	2.63889	1.000	2.15278	1.000

		Group II	10.76923*	.002	3.36538	1.000
		Group III	14.90385*	.000	5.28846	.603
	Group I	Group IV	16.63462*	.000	7.50000	.161
		Group I	-10.76923*	.002	-3.36538	1.000
		Group III	4.13462	.519	1.92308	1.000
	Group II	Group IV	5.86538	.127	4.13462	1.000
		Group I	-14.90385*	.000	-5.28846	.603
		Group II	-4.13462	.519	-1.92308	1.000
	Group III	Group IV	1.73077	1.000	2.21154	1.000
		Group I	-16.63462*	.000	-7.50000	.161
		Group II	-5.86538	.127	-4.13462	1.000
HDL	Group IV	Group III	-1.73077	1.000	-2.21154	1.000
		Group II	-10.76880*	.003	-.09615	1.000
		Group III	-16.05705*	.000	-14.80710*	.000
	Group I	Group IV	-18.94155*	.000	-15.96090*	.000
		Group I	10.76880*	.003	.09615	1.000
		Group III	-5.28825	.241	-14.71095*	.000
	Group II	Group IV	-8.17275*	.024	-15.86475*	.000
		Group I	16.05705*	.000	14.80710*	.000
		Group II	5.28825	.241	14.71095*	.000
	Group III	Group IV	-2.88450	1.000	-1.15380	1.000
		Group I	18.94155*	.000	15.96090*	.000
		Group II	8.17275*	.024	15.86475*	.000
TC	Group IV	Group III	2.88450	1.000	1.15380	1.000
		Group II	-11.48000*	.001	-11.91500*	.002
		Group III	-17.31750*	.000	-19.84500*	.000
	Group I	Group IV	-28.21500*	.000	-28.53250*	.000
		Group I	11.48000*	.001	11.91500*	.002
		Group III	-5.83750	.072	-7.93000*	.034
	Group II	Group IV	-16.73500*	.000	-16.61750*	.000
		Group I	17.31750*	.000	19.84500*	.000
		Group II	5.83750	.072	7.93000*	.034
	Group III	Group IV	-10.89750*	.001	-8.68750*	.019
		Group I	28.21500*	.000	28.53250*	.000
		Group II	16.73500*	.000	16.61750*	.000
ALT	Group IV	Group III	10.89750*	.001	8.68750*	.019
		Group II	-20.90750*	.001	-22.30250*	.000
		Group III	-42.94500*	.000	-41.13250*	.000
	Group I	Group IV	-67.21250*	.000	-68.12250*	.000
		Group I	20.90750*	.001	22.30250*	.000
		Group III	-22.03750*	.001	-18.83000*	.001
	Group II	Group IV	-46.30500*	.000	-45.82000*	.000
		Group I	42.94500*	.000	41.13250*	.000
		Group II	22.03750*	.001	18.83000*	.001
AST	Group III	Group IV	-24.26750*	.000	-26.99000*	.000

		Group I	67.21250*	.000	68.12250*	.000
		Group II	46.30500*	.000	45.82000*	.000
	Group IV	Group III	24.26750*	.000	26.99000*	.000
		Group II	-.43500	1.000	-.93000	.057
		Group III	-1.14000*	.024	-.73500	.188
	Group I	Group IV	-1.23000*	.014	-1.24500*	.008
		Group I	.43500	1.000	.93000	.057
		Group III	-.70500	.289	.19500	1.000
	Group II	Group IV	-.79500	.174	-.31500	1.000
		Group I	1.14000*	.024	.73500	.188
		Group II	.70500	.289	-.19500	1.000
	Group III	Group IV	-.09000	1.000	-.51000	.700
		Group I	1.23000*	.014	1.24500*	.008
		Group II	.79500	.174	.31500	1.000
Urea	Group IV	Group III	.09000	1.000	.51000	.700
		Group II	-.19383*	.000	-.16106*	.000
		Group III	-.26414*	.000	-.23249*	.000
	Group I	Group IV	-.28054*	.000	-.25291*	.000
		Group I	.19383*	.000	.16106*	.000
		Group III	-.07031*	.003	-.07144*	.001
	Group II	Group IV	-.08671*	.000	-.09186*	.000
		Group I	.26414*	.000	.23249*	.000
		Group II	.07031*	.003	.07144*	.001
	Group III	Group IV	-.01640	1.000	-.02042	.878
		Group I	.28054*	.000	.25291*	.000
		Group II	.08671*	.000	.09186*	.000
Creatinine	Group IV	Group III	.01640	1.000	.02042	.878

Declaration

This is to certify that the thesis prepared by Hailemariam Amsalu entitled **The Effect of Palm Oil Fried Street Kokor on Lipid Profiles, Liver and Kidney Histopathology and Biomarkers of Swiss Albino Mice** and submitted in the partial fulfillment of the requirements for the Degree of Master of Science in Medical Biochemistry complies with regulation of Jimma University and meets the accepted standards with respect to originality and quality.

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