Evaluation of the Effect of *Lepidium sativum L.* Seed Extract on Lipid Profile, Liver Function Tests and Liver Histopathology of Male Swiss Albino Mice Fed on Deep Fried Palm Oil Diet

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Declaration

This is to certify that the thesis prepared by Ebsa Tofik entitled: Evaluation of the Effect of *Lepidium sativum L.* Seed Extract on Lipid Profile, Liver Function Tests and Liver Histopathology of Male Swiss Albino Mice Fed Deep Fried Palm Oil Diet submitted in partial fulfillment of the requirements for the Degree of Master of Science in Medical Biochemistry complies with regulation of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Background: Deep frying involves immersing food completely in hot oil with contact among oil, air and food moisture at a temperature of 150 to 200°C. It is not only limited to homes, but also practiced highly among street food vendors, and restaurants. However, most people often keep and reuse the oil since deep frying process requires a high amount of oils. Reused fried palm oil has been shown to affect lipid profile, liver function tests and liver histopathology. Therefore, the current study was aimed to investigate the effect of *Lepidium sativum L.* seed extract on the lipid profile, liver function tests and liver histopathology of male Swiss albino mice fed deep-fried palm oil diet.

Methods: The random posttest only control group experimental design was performed. Twenty four male Swiss albino mice of age 8 to 10 weeks and weight 32–42 g were divided into four groups. Group I was fed on normal basal diet and distilled water. Group II was fed the deep fried palm oil diet and distilled water. Group III and IV were fed deep fried palm oil diet and LSSE at a dose of 200 and 400 mg/kg/day, respectively for 28 days. Then on 29th day, the mice were fasted overnight, anesthetizing with diethyl ether, 2-2.5 ml of the blood was taken by cardiac puncture and sacrificed by cervical dislocation. Blood was used for lipid profile and liver function tests while liver tissues were taken for histopathology investigation. Qualitative analysis were carried out by Senior Pathologist for each group and presented in the form of photomicrography. The quantitative data were entered to the epi-data version 3.1 and SPSS version 25 was used for data analysis. ($p < 0.05$) were considered to be statistically significant.

Results: The serum lipid profile TC, and LDL was decreased significantly ($p < 0.05$) while the HDL increased significantly ($p < 0.05$) in group IV. However, the serum TG levels showed a significant ($p < 0.05$) decrement in both group III and IV. The serum ALT and total bilirubin showed significant ($p < 0.05$) decrement whereas the serum albumin levels showed significant ($p < 0.05$) increment in group IV. However, the serum AST and ALP level was decreased significantly ($p < 0.05$) in both group III and IV. The group IV at 400mg/kg/day liver section of mice showed better effect of LSSE on restoring liver histopathology towards normal.

Conclusion: The present study result revealed that the LSSE were effective on the lipid profile and liver function that altered by feeding deep fried palm oil diet.

Keyword: *Lepidium sativum,* Deep fried palm oil, Liver function tests, Lipid profile, Liver Histopathology, Swiss albino mice.

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ABBREVIATIONS AND ACRONYMS

ALT	Alanine Amino Transferase
ANOVA	Analysis of Variance
AST	Aspartate Amino Transferase
CHD	Coronary Heart Disease
CVD	Cardiovascular Disease
DPX	Dibutyl Phthalate Xylene
GIT	Gastro Intestinal Tract
HDL	High Density Lipoprotein
HFD	High fat diet
HMG-CoA	Hydroxymethylglutaryl Coenzyme A
LCAT	Lecithin: Cholesterol Acyl Transfarese
LFTs	Liver Function Tests
LSSE	*Lepidium sativum* seed Extract
MUFA	Monounsaturated Fatty Acid
NCDs	Noncommunicable Disease
NIH	National Institutes of Health
PUFA	Polyunsaturated Fatty Acid
ROS	Reactive Oxygen species
SFA	Saturated Fatty Acid
SPSS	Statistical Package for Social Science
SR-B1	scavenger receptor class B type I
SST	Serum Separator Tube
TC	Total Cholesterol
TIDRC	Tropical and Infectious Disease Research Center
TPO	Thermooxidized Palm Oil
WHO	World Health Organization

1. INTRODUCTION

1.1 Background

Frying is one of the most common methods of food preparation worldwide. During frying process the oil is exposed continuously to a high temperature in the presenc of air and food moisture. Frying methods vary based on the amounts of oil used, the way of handling food and cooking times (1). Deep frying is one of the most widely used frying methods. It involves immersing food completely in hot oil with contact among oil, air and food moisture at a temperature of 150 to 200℃ (2). It is not only limited to homes, but also practiced regularly in amongst street food vendors, restaurants and commercial food industry. These are due to operational simplicity, economic affordability, and ability to improve sensory characteristics in fried foods that are highly appreciated by the consumer (3).

Vegetable oils are a major component of daily fried food intake and its global use has increased highly since 1980. Palm oil is one of the edible vegetable oil, which obtained from the mesocarp fruits of the tropical *Elaeis guineensis.* It represents 30% of the world's vegetable oil production and three- quarters of which are used for food preparation. Moreover, its consumption has increased by tenfold and these constitutes around one-third of global vegetable oil consumption (4,5).

Palm oil contains almost 50% saturated fatty acids (SFA), 50% monounsaturated fatty acids (MUFA) and low levels of polyunsaturated fatty acids (PUFA) under normal conditions. Besides, it contains carotenoids, tocopherols and tocotrienols as important antioxidants which intensify its oxidative stability in combination with other chemical compositions. These chemical compositions and low in price amongst dietary oils enhance high percentages of palm oil to use for frying (6,7).

However, like other edible oil it undergoes various physical and chemical reactions during the deep-frying process. A physical reaction involves the formation of foam, increases in viscosity, darkening of color, and deterioration of flavor. These changes affect the sensory qualities of fried foods such as odor and taste of the oil. Chemical reactions include hydrolysis, isomerization, polymerization and oxidation (8,9).

Hydrolysis is breakage of triglycerides through deep frying and production of monoglycerides, diglycerides, free fatty acids and glycerol. Isomerization causes configuration of the fatty acids to change from the *cis* isomer to the *trans* isomer. Polymerization produces high molecular cyclic fatty acid monomers, triglyceride dimmer (TGD) and triglyceride oligomer (TGO). Oxidation causes triglyceride molecules in the frying oil to undergo primary oxidation and form unstable lipid species such as peroxides and hydroperoxides. These react rapidly with each other to form secondary oxidation products that comprise non-volatile and volatile compounds. The non-volatile polar product includes carbonyls, dimeric, trimeric, polymeric, fatty acids and volatile compounds include alcohols, aldehydes, acids, and ketones. In combination, these oxidation products affect the chemical compositions of the palm oils by oxidizing its fatty acids, decreasing antioxidant (vitamin E) contents, produce polar and polymeric products (8,10).

The products formed during deep frying deposits in frying oils and absorbed by the fried food. After consumption of fried food, it enters into the gastrointestinal tract (GIT) and blood circulation where it damages endothelial cells and lipids by initiating lipid peroxidation. Lipid peroxidation is the oxidative deterioration of lipids in which the oxidants, such as free radicals or non-radical species attack the carbon-carbon double bonds of the membrane and circulating lipids (11). The low density lipoprotein (LDL) oxidized easily from the circulating lipids because of their high PUFA content and the oxidatively modified LDL are more avidly taken up by macrophages via the scavenger receptor than native LDL. These cause deposition of lipid in the endothelial space that leads to atherosclerosis process that produces an atherosclerotic plaque and cardiovascular disease (CVD) (12,13).

Cardiovascular disease (CVD) affects the functions of the heart and blood vessels. It includes hypertension, coronary heart disease (CHD) and stroke which cause is linked to the complications of atherosclerosis. Atherosclerosis is a chronic disorder of large and medium-sized arterial wall characterized by endothelial dysfunction, vascular inflammation, and lipid deposition in the intima (14). In addition to alteration of the lipid, the previous studies also revealed that the administration of deep frying oil can cause damage to hepatocyte membrane and integrity that manifested through raised liver damage markers and altered histopathology of the liver (15,16).

However, consumption of vegetables and fruits play a key role in the promotion and maintenance of good health (17). *Lepidium sativum* is one of the most important vegetables and herbal medicines. In Ethiopia, the *Lepidium sativum L.* seed traditionally used for treating skin problems, eye diseases, amoebic dysentery, abortion, asthma, intestinal complaints, gastritis, ringworm, malaria, tonsillitis and stomach ache (18,19). It is also used to treat hypertension, liver diseases, and jaundice. Furthermore, its seed is one of the functional foods and that contains ingredient such as saponin, flavonoids, alkaloids, terpenoids and steroids which have antioxidant, antiatherosclerotic, and hepatoprotective capacity as supported by different literature (20–22). However, even though there is reported scientific data pertaining to *Lepidium sativum* seed effect on lipid profile, liver function tests and liver histopathology in other countries with different experimental designs such studies are lacking in our countries. Therefore, this study aimed to investigate the effect of LSSE on the lipid profile, liver function tests and liver histopathology of male Swiss albino mice fed deep fried palm oil diet.

1.2. Statement of the Problem

The rapid urbanization, mechanization and economic development of Africa and the world at large have resulted in a dietary transition from a traditional to a modernized diet, where the quality of food has been affected. Currently, Ethiopia is also undergoing an epidemiologic transition mainly driven by demographic and lifestyle changes that promotes enormous changes in diets. Deep fried foods are gaining more popularity due to their increased durability and desirable sensory characteristics. These resulted in extensive sales of a variety of fried foods and rapid expansion of deep frying practices (3,23,24).

However, a major drawback is as the process of deep-frying often needs a large amount of oils people most often keep the used frying oil for reuse to ensure cost-effectiveness. In addition to cost, the underlying reason for reusing repeatedly deep fried oils are due to low level of awareness among the public about its negative effect on health (25). The palm oil undergoes chemical reactions during the deep frying process that resulted in the formation of oxidative products. These affect the lipid metabolism which in turn results in alteration of the normal lipid profile as the study revealed it mostly on experimental animals. The previous study on the effect of boiled oils on lipid profile showed an increase in serum total cholesterol, LDL, triglycerides (TG) levels but decreased in high-density lipoprotein (HDL) level in the animals treated with repeatedly boiled oils when compared to control group (26).

The elevated plasma LDL while decreased HDL are associated with atherosclerosis which are primary risk factors for CVD. The burden of CVD has grown disproportionally in low and middle-income countries in the past few decades. The high burdens of CVD in developing countries are attributable to the increasing incidence of atherosclerotic diseases (27,28). CVD is one of the major causes of morbidity and mortality worldwide from the non-communicable diseases (NCDs). NCDs are the leading cause of death across the world. It accounts for 73.4% of all deaths globally according to the Global Burden of Disease (GBD) study in 2017. It is the second most common cause of death in sub-Saharan Africa (SSA) that accounts for 2.6 million deaths. In Ethiopia, approximately 42% of all deaths are due to NCDs, of which 27% are premature deaths (29,30). Even though there are many synthetic drugs on the market for the clinical treatments of serum lipid profile disorder, the statins are widely used drugs because of its significant effectiveness in lowering the level of LDL. However, statin consumption results in adverse effects such as liver damage, muscle toxicity, myopathy and acute kidney failure (31,32).

Moreover, the oxidative product, especially lipid peroxidation products, induce damage to the liver that account for the loss in liver normal function and leakage of its content. The study conducted in Nigeria on the repeatedly heated palm oil fed rat revealed that the damage to liver normal function through decreased serum albumin concentration and leakage of increased liver damage markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin into the circulation (33).

Even though a liver problems due to several causes are one of the huge life-threatening diseases worldwide and many drugs are available for liver diseases, they do not give satisfactory results due to adverse effects and high cost (34). Thus, the side effects of synthetic drugs and their high cost in treating lipid profile disorder and liver disease have increased the need to look for herbal medicine that are in the form of dietary supplementation and rich in phytoconstituents that restore lipid profile and liver markers to normal.

Therefore, it is important to develop alternative methods of improving such health benefits with less adverse effects, more cost effective, locally available, and easily consumable to provide better safety and efficacy on a long term usage treatment agents. One of the most important herbal medicines is *Lepidium sativum L.*and this study aimed to investigate the effect of *Lepidium sativum L* seed (collected from local markets) on lipid profile, liver function tests and liver histopathology on male swiss albino mice fed in deep fried palm oil diet.

1.3. Significance of the Study

In Ethiopia, *Lepidium sativum L.* seed effect on lipid profile and liver is not exhaustively reported. Therefore, this study finding will aid in reporting scientific data regarding to effect of *Lepidium sativum L.* seed on lipid profile and liver function. *Lepidium sativum L.* seed is commonly available in local areas. Moreover, it's very cost effective; food based herbal medicine with fewer side effects. But it is utilization in the study community is under its health benefits. Hence, this study finding help to reveal the importance of consumption of *Lepidium sativum L.* seed as a part of their routine, diet, especially along with deep fried product. Furthermore, there is no more study in this particular area and in general in Ethiopia, on the effect of *Lepidium sativum L.* seed extract on lipid profile, liver function tests and liver histopathology of male Swiss albino mice fed deep fried palm oil diet. Thus, the findings of this study will provide baseline input for further research.

2. LITERATURE REVIEW

2.1 Lipid Profile

Lipids are water-insoluble cellular components of diverse structure. It includes a family of compounds such as triglycerides, phospholipids, and sterols. Cholesterol is the major sterol in animals that obtained from the diet or may be produced endogenously and it is an essential substance that involved in many functions such as the precursor of steroid hormones, bile acids, vitamin D and regulates membrane fluidity. Lipoproteins are complexes of amphipathic proteins with lipids at variable ratios, densities, and sizes which transport water-insoluble lipids in the blood. Lipoproteins have been grouped into five major classes based on their buoyant density: chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).Some of these are the important parameters for measurement of the serum lipid as lipid profile (35,36).

Lipid profile is well-established predictors of vascular disease. The most frequently measured lipid profile is TC, HDL, LDL and TG. The most common lipid abnormality was low HDL cholesterol, and elevated levels of LDL cholesterol (27,37). HDL is responsible for peripheral tissue cholesterol uptake and delivery to the liver where converted into bile acids for excretion or directly secreted into bile. It facilitates transfer to the liver via scavenger receptor class B type I (SR-B1), after cholesterol uptake from peripheral tissue. It is associated with apolipoprotein A (Apo A) that facilitates cholesterol transfer from peripheral tissue and activates lecithin-cholesterol acyltransfarase (LCAT). LCAT allows cholesterol esterification and movement of cholesterol from the HDL surface to the HDL core. Increased HDL provides important antioxidant and anti-inflammatory functions that can inhibit atherosclerosis (38).

LDL produced from VLDL following the removal of triglyceride and responsible for cholesterol transport to peripheral tissue where it is used either in membranes or for the synthesis of steroid hormones. Cells take up cholesterol by receptor-mediated endocytosis when LDL binds to its receptor and internalized in an endocytic vesicle. Then, receptors are recycled to the cell surface whereas hydrolysis in an endolysosome releases cholesterol for use in the cell. High levels of LDL cholesterol increase the risk for atherosclerosis highly because LDL particles play important role in the formation of atherosclerotic plaques (39,40).

Liver is a major organ that play important role in regulation of cholesterol levels in the body. It does not only play a role in synthesizing cholesterol for export to other cells ,but it also removes cholesterol from the body through converting it to bile salts and putting it into the bile where it can be removed in the feces. Besides, the liver synthesizes the lipoproteins involved in transporting cholesterol and lipids throughout the body. Regulation of cholesterol is mainly centered on the metabolism of lipoproteins that mediate transport of the lipid to and from tissues (41,42).

2.2. Effect of Deep Fried Palm Oil on Lipid Profile

Deep frying of oils causes the formation of geometrical isomers of fatty acids**.** It causes a change in the configuration of the fatty acids from *cis* to the *trans* isomer. Many *trans* fatty acids can be formed during reusing the oil many times under high temperature. *Trans* fatty acids harm blood lipids by increasing LDL and decreasing HDL, which have been shown to increase the risk of heart disease. It increases LDL similarly to saturated fat but unlike saturated fat, they also decrease HDL cholesterol (25). As a result, the net effect of *trans* fat on cholesterol is approximately double to that of saturated fat. The risk of cardiovascular disease is increased if *trans* fatty acids are consumed in high amounts. Previous metabolic and epidemiological studies have reported that consumption of *trans* fatty acids confers increased risk of cardiovascular disease due to changes in blood lipid levels. The study reported that repeatedly heated palm oil increases lipid peroxidation and total cholesterol thereby increasing the risk of atherosclerosis which exerts adverse effects on health (13).

Previous research findings revealed altered lipid profiles that promote atherosclerosis and CVD in mice fed repeatedly deep fried palm oil. The study conducted in Malaysia by Farida; concludes that the usage of repeatedly heated palm oil is the predisposing factor for atherosclerosis and leading to cardiovascular diseases (43).

2.3. Liver Function Tests

Liver is the largest organ of the human body having a broad range of functions. It has numerous functions in the body including metabolism, detoxification, excretion, and synthesis of protein. It is susceptible to exogenous substances such as drugs, alcohol and environmental toxins, which can lead to liver disorders (34,44).

Liver function tests (LFTs) are useful screening tools that are an effective approach to detect hepatic dysfunction. LFTs most commonly include ALT, ALP, AST, total bilirubin and albumin (45). ALT is mainly aggregated in the cytosol of the liver cell. Its activity in hepatic cells is higher than serum ALT activity. When liver damage occurs, it is released from damaged liver cells and causes a significant elevation in serum. It is also found in muscles, adipose tissues, intestines, colon, prostate, and brain, however, its concentration in these organs is much lower than the liver. Thus, the estimation of this enzyme is more specific for detecting liver abnormalities since it is present in the liver at higher concentrations than other organs. AST play important role in proteins metabolism. In addition to liver, it is also found in other organs like heart, muscle, brain and kidney. The damage to any of one these tissues induce its increment in blood. Thus, it is considered a less specific biomarker enzyme for hepatocellular injury since it can also signify abnormalities in other organs like heart, muscle, brain and kidney (46,47).

Alkaline phosphatase is a hydrolase enzyme that is eliminated in the bile and hydrolyzes monophosphates at an alkaline pH. It is particularly present in the cells, which line the biliary ducts of the liver and also found in other organs including bone, placenta, kidney and intestine. It may be elevated if bile excretion is inhibited by liver damage. Liver damage leads to elevation of the normal values due to the body's inability to excrete it through bile due to the congestion or obstruction of the biliary tract, which may occur within the liver, the ducts leading from the liver to the gallbladder, or the duct leading from the gallbladder through the pancreas that empty into the duodenum (48).

Albumin is a globular, water-soluble protein with negatively charged at neutral pH which attributed due to the abundance of aspartate and glutamate residues in its sequence. It is the most abundant protein in plasma which represents half of the total protein content of plasma in healthy humans. It is exclusively synthesized by hepatocytes (49,50). It functions as transporter of variety of substances such as bilirubin, ions, fatty acids, and exogenous drugs. Its synthesis is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure (50,51).

Bilirubin is an endogenous anion derived from degradation of hemoglobin and excreted from the liver in the bile. Hemoglobin is derived from red blood cells that have outlived their natural life. During splenic degradation of red blood cells, hemoglobin is separated out from iron and cell membrane components. Normally present in the blood in small amounts and used by the liver to produce bile. When the liver cells are damaged, then not able to excrete bilirubin in the normal way, these cause increased bilirubin in the blood. Increased levels of bilirubin may also result due to decreased hepatic clearance (46,52).

2.4. Effect of Deep Fried Palm Oil on Liver

Repeated heating of palm oil at high temperature decreases the antioxidant content in the oil and these enhance the formation of free radicals that remain deposited in the frying oils. Peroxides and hydroperoxides are among the most commonly produced free radicals that responsible for the decrease of antioxidant defense, propagation of lipid peroxidation and oxidative stress development. The previous study also reported the increased oxidative stress and lipid peroxidation in the liver of rat that fed repeatedly heated oil (53,54).

Oxidative stress promotes lipid peroxidation of the hepatocyte membrane that in turn involved in liver membrane damage and causes the release of serum transaminases such as AST, and ALT into the circulation (10). The levels of these enzymes are widely used in the assessment of liver injury. They are indicative of cellular leakage and loss of functional integrity of the cell membranes in the liver when their levels are elevated in the serum (55). ALP is also another indicator of liver damage. When the liver is damaged it leaks out from the liver and its concentration increase in serum especially in the case of the advanced stage of liver damage like bile duct damage, and obstruction of the bile duct (56).

The consumption of repeatedly boiled oil in the diet induces decrease in serum albumin level through triggering nutrient deficiencies through thermal destruction of essential vitamins and fatty acids in the oxidized oil which combined with decreased protein digestibility and absorption due to cross-linking reactions of secondary lipid oxidation products with proteins (26). Besides, repeatedly frying of palm oil also results in the generation of cytotoxic and destructive by products which are injurious to cells, tissues and organs. Previous animal studies showed that ingestion of food containing thermooxidized palm oil (TPO) resulted in inflammation and microsteatosis changes in the liver (57).

2.5. *Lepidium sativum L.*

The traditional medicinal uses have witnessed an upsurge of interest in developing and developed countries in the last few decades. In Ethiopia also plants have been used as a source of medicine from time immemorial to treat different ailments and become an integral part of the culture. About 80% of the human population and 90% of livestock depend on herbal medicines in our country (58,59).

Lepidium sativum L. are an edible annual herb which belongs to the *Brassicaceae* family. It is an erect, glabrous, herbaceous plant growing up to 15-45cm in height with small white flowers in long racemes (55).It has different names in different languages. It is named as garden cress in English, fetto in Amharic, shimfi in Afaan-Oromo, Shimfa in Tigrigna, Shimp in Kaffa and Shufu in Adere language (19). Seed are smooth, small and reddish brown. The shape is oval with point and triangular at one end. Seed length is about 3-4 mm and width is 1-2 mm (60,61). There is a white scar near the point of attachment, from which a small channel extends to 1/3 the length of the seeds. Seeds are odourless and the taste is pungent (55).

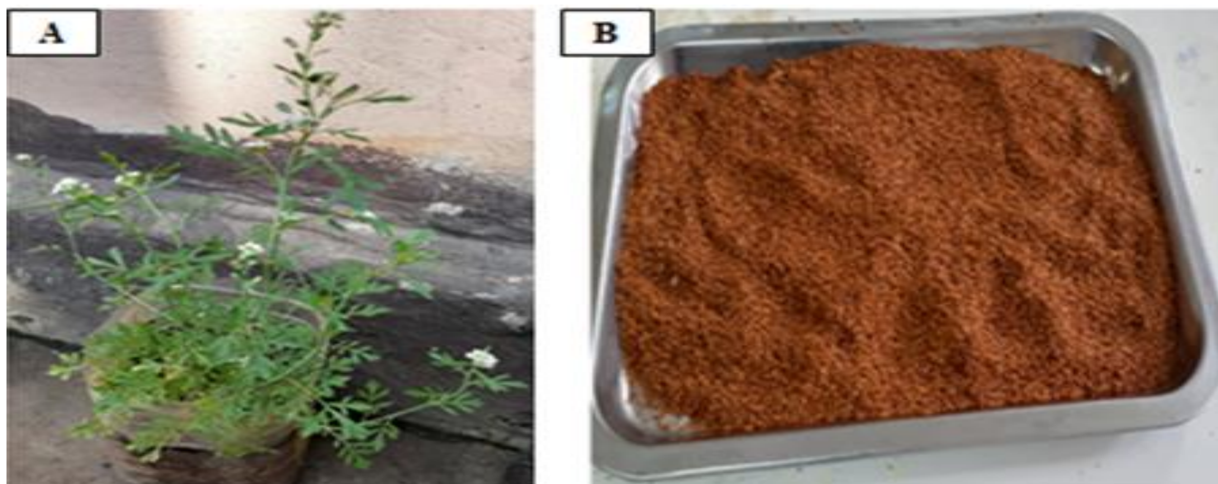


Figure 1 : *Lepidium sativum L.* plant (A), and seed (B) (photo by PI)

2.6. Phytochemistry and Pharmacological Effects of *Lepidium sativum L.*seed

Analysis of *Lepidium sativum* seed showed that the seed contains proteins, fats, crude fiber, essential amino acids, and carbohydrates. It also contains 24% oil which composed mainly of α -linolenic acid (ALA) (32%) and linolenic acid (LA) (12%) (21).

The *Lepidium sativum* seed also contains vital minerals constituents like calcium, magnesium, phosphorus, iron, potassium, sodium, zinc, and vitamins mainly tocopherol, thiamine, riboflavin, and niacin (62–64). The phytochemical screening of *Lepidium sativum* seed showed that it contained glycoside, alkaloids, flavonoids, saponins, sterols, tannins, and triterpene (65). Additionally, the quantitative analysis of *Lepidium sativum* seeds showed that the seeds contained alkaloids (0.40%), flavonoid (0.42%), saponin (2.8%), and tannin (0.61%) (3, 25, 66).

Flavonoids are an organic compound that is diverse in chemical structure and characteristics. Studies have shown that flavonoids are responsible for a variety of pharmacological effects due to their high antioxidant activity in both *in vivo* and *in vitro* systems. The antioxidant activity action includes suppression of reactive oxygen species (ROS) formation by either inhibition of enzymes or chelating trace elements involved in the free radical generation (67,68). The various therapeutic actions of *Lepidium sativum* seed on different chronic diseases also imparted by its important set of flavonoid composition. The previous study reported the concentrations of six major flavonoids in *Lepidium sativum L.* seed as follows naringin (12.4mg/g), quercetin (2.81mg/g), naringenin (24.87mg/g), luteolin (2.34mg/g), kaempferol (1.82mg/g), and apigenin (0.95 mg/g) (69).

Naringenin is a predominant flavanone found in the methanolic extract of the *Lepidium sativum* seed. It is endowed with broad biological effects on human health that includes a decrease in lipid peroxidation products, protein carbonylation, increases antioxidant defenses, scavenges reactive oxygen species, anti-atherogenic and anti-inflammatory effects. Naringenin was shown to reduce circulating levels of low-density lipoprotein (LDL) by 17% in hypercholesterolemic patients (70). The previous study reported that naringenin at a 0.1% dietary level lowered levels of plasma cholesterol in rats fed a high-cholesterol diet by a decreasing the activity of 3-hydroxy-3-methylglutaryl coenzyme A (CoA) reductase. Naringenin supplementation also restored serum albumin concentrations in dimethylnitrosamine induced hepatotoxicity in rats (70,71).

Naringin is a flavanone found in grapes, citrus fruits and herbs like *Lepidium sativum* seed. In the previous study, like naringenin, its supplementation also lowered elevated plasma lipid and cholesterol concentrations in high fat diet fed rats. The hepatic 3-hydroxy-3-methyl CoA (HMG-CoA) reductase activity was significantly reduced in the naringin-supplemented (0.02 g/100 g) group of mice (71,72). Hepatoprotective actiozn of naringin was reported by several studies. Its supplementation significantly lowered the elevated plasma transaminase activity in nickel and cadmium induced liver toxicity in rats (73,74).

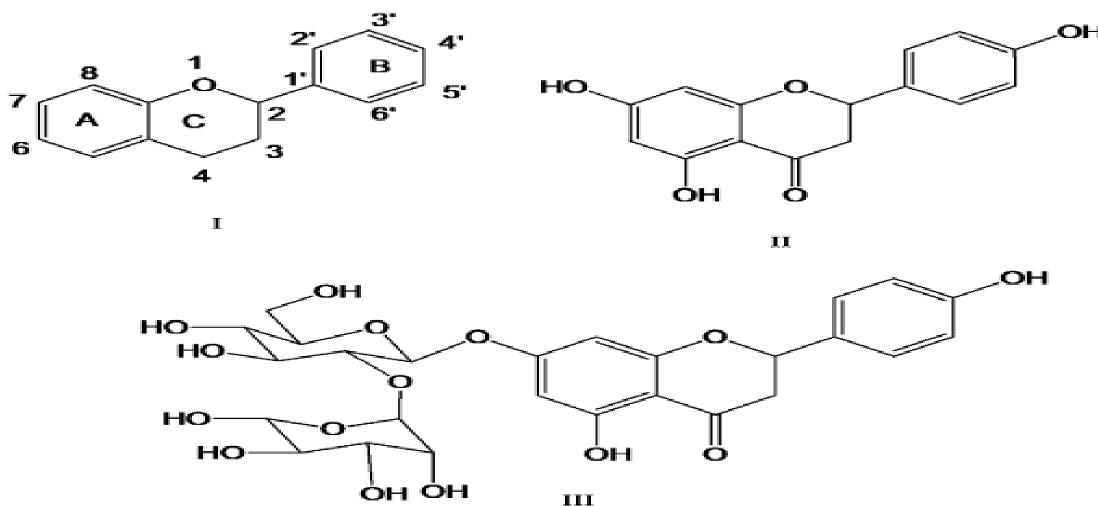


Figure 2: Basic structure of flavonoids (I), naringin (II), and naringenin (III) (71)

2.7. Conceptual Framework

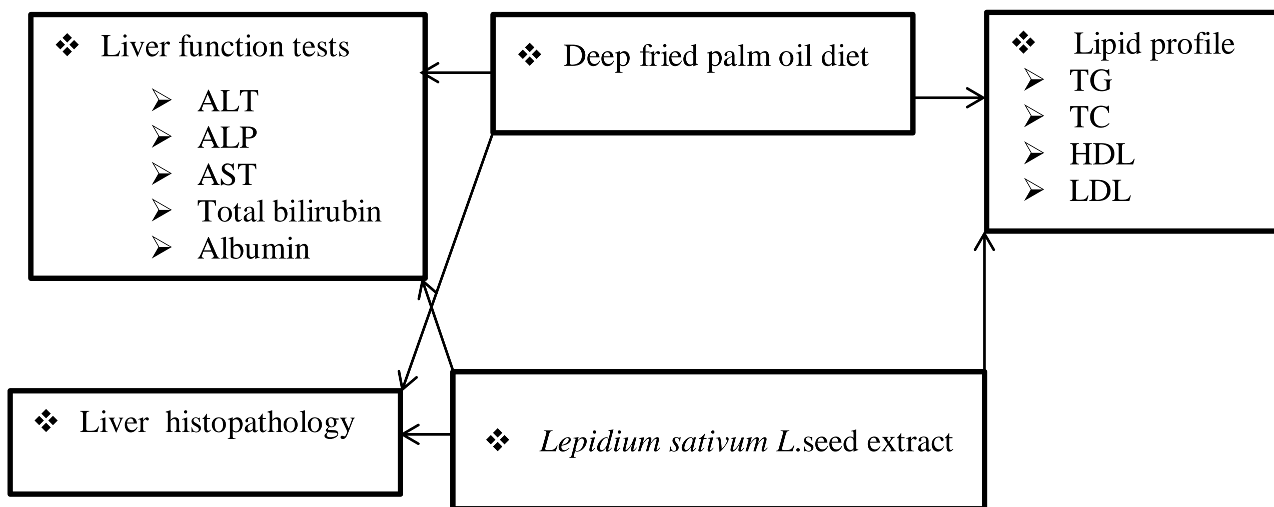


Figure 3 : Conceptual frame work (derived from different literatures)

3. OBJECTIVES

3.1. General Objective

To investigate the effect of *Lepidium sativum L.* seed extract on lipid profile, liver function tests and liver histopathology of male swiss albino mice fed on deep fried palm oil diet

3.2. Specific objectives

- To conduct preliminary phytochemical screening of *Lepidium sativum L.* seed using different organic solvent
- To determine the effect of *Lepidium sativum L.* seed extract on lipid profile (TC,HDL,LDL and TG) of male Swiss albino mice fed on deep fried palm oil diet
- To evaluate the effect of *Lepidium sativum L.* seed extract on liver enzymes (ALT,AST and ALP); serum Albumin and Total bilirubin; and liver histopathology of male Swiss albino mice fed on deep fried palm oil diet

Hypothesis of the Study

Null Hypothesis (Ho): *Lepidium sativum L.* seed extract has no effect on lipid profile, liver function tests and liver histopathology of male Swiss Albino Mice fed deep fried palm oil diet

Alternative Hypothesis (HA): *Lepidium sativum L.* seed extract has an effect on lipid profile, liver function tests and liver histopathology of male Swiss Albino Mice fed deep fried palm oil diet

4. METHODS AND MATERIALS

4.1. Study Area and Period

The study was conducted at a street food vendors; Organic Chemistry and Veterinary medicine post graduate laboratores of Jimma University. The experiment on Male Swiss Albino Mice was conducted from July 28 to August 27/2020.

4.2. Study Design

The random posttest only control group experimental design was performed.

4.3. Experimental Animals

A total of twenty four male (24) mice of age 8 to 10 weeks and weighed 32–42 g were used for investigating the effect of *Lepidium sativum L.* seed extracts on lipid profile, liver function tests and liver histopathology. The female mice were excluded from the study due to hormonal variation (estrogen hormone) that confound with plant extract effect. All the mice were obtained from the Tropical and Infectious Disease Research Center (TIDRC), Sokoru, Jimma, South western Ethiopia. Accordingly, it was brought to Veterinary Medicine Postgraduate Laboratory and had free access to standard mice pellet and distilled water in accordance to the National Institutes of Health (NIH) Guidelines for Care and Use of Laboratory Animals (75). The mice were housed in a transparent plastic cage with dimensions of length 40 cm, width 20 cm, height 15 cm and with SS sipper 250 ml water bottle at room temperature and 12h light/dark cycle. Wood shaving was used as bedding and it was replaced every morning after the cage was cleaned. Besides the wood shaving, the mice food (pellet) and distilled water were replaced by fresh one every morning. The mice were allowed to acclimatize to the laboratory environment for seven (7) days before being subjected to the experiments.

4.4. Sample Size Determination

The sample size was determined using the Federer formula which was $(t-1)(n-1) \geq 15$, whereas t was the number of the groups and n was the experimental animal per group (76). The study was performed using four (4) groups of mice. Therefore, the total sample size was 4 groups × 6 mice = 24 mice.

4.5. Animals Grouping and Dose Administration

Animal grouping and dose administration was conducted in Veterinary Medicine postgraduate laboratory, College of Agriculture and Veterinary Medicine, Jimma University. Twenty four male Swiss albino mice were divided randomly into four groups that contain six in each cage. Each mouse in the given group was differentiated by giving a number on its tail by a permanent marker. At the beginning of the experiment and then weekly, mouse body weight was measured by electronic balance to adjust the *Lepidium sativum* seed extract (LSSE) dose administration. Administration of *Lepidium sativum* seed extract dose was based on the methanolic extract of the *Lepidium sativum* seed that was safe up to 2000 mg/kg. Thus, based on this the initial dose for this study start from the 10 %(200mg/kg/day) of the 2000mg/kg and the second dose was determined by taking the double of the initial dose(400mg/kg/day) (77). Each dose was orally administered with oral gavage needle (18G, 2.5 cm in length) daily for a period of 28 days.

Table 1 : Animal Grouping

Groups	Category	Dose Administration
I	Normal control (c_0)	Normal Mice Pellet + 2ml/100g Distilled Water Orally
II	Negative control (c_1)	DFPOD + 2ml/100g Distilled Water Orally
III	Treatment One (T_1)	DFPOD + 200 mg/kg LSSE
IV	Treatment Two (T_2)	DFPOD + 400mg/kg LSSE

The letter indicates: C=Control, T=Treatment, DFPOD= Deep fried palm oil diet, LSSE=*Lepidium sativum* seed extract

4.6. Chemical and Reagents

In the present study the following chemicals were used: (80% V/V) methanol (Sigma Alderich, UK) was used for extraction of *Lepidium sativum* seed, iodine, potassium iodide, chloroform, acetic acid, sulphuric acid, sodium hydroxide, hydrochloric acid, 5% ferric chloride reagents were used for phytochemical screening, eosin was used to stain hepatocyte cytoplasm, Dibutyl Phthalate in Xylene (DPX), hematoxylin were used for staining hepatocyte nucleus, di-ethyl ether was used for anesthetizing mice, distilled water, liver function test reagents, (70%,80%,90%,100%) ethanol were used both for tissue dehydration and hydration, formalin

(10%) was used for tissue preservation, paraffin wax was used for making the tissue hard enough for cutting and xylene used as cleaning agent to remove both the dehydrating agent (alcohol) and paraffin wax.

4.7. Equipment

In this study, rotavapor (Buchi Rota Vapor, Switzerland) was used for filtrate separation, What man filter paper No.1(Whatman®, England), test tube, beakers, funnels, Erlenmeyer flasks, , spatula, stirrer, centrifuge (Gemmy Industrial Corp, Taiwan) was used for serum separation, aluminum foil, oral feeding blunt needles, scissors, refrigerator, balance (Mettler Toledo MS104S, Switzerland), forceps, slides, cover slides, tissue processing cassettes, open tissue processor (LEICA TP 1020,Germany), microtome blade(LEICA RM 2125 RTS,Germany), oven (Heraeus, D6450 Hanau), deep fat fryer (Oppein model-OP-81, China) was used for the preparation of the deep fried palm oil, mortar and pestle was used for the preparation of the coarse seed powder, lyophilizer (Alpha 1-2 LD plus, John Morris scientific pvt. Ltd) was used to remove the water from the filtrate, stainless steel and plastic cage, SS sipper 250 ml water bottle, microscope and vegetable slicer, and SD Lipido Care ® analyzer(SD Biosensor, INC, Korea) ABX Pentra 400 clinical chemistry analyzer (China).

4.8. Plant Material Collection and Preparation

Lepidium sativum L. seed was purchased from Jimma market, in April 2020. Then, the plant was authenticated by plant taxonomist Mr. Melaku Wondafrash at the National Herbarium of Addis Ababa University. The voucher number of the plant (ET-01/2020) was given. The plant material preparation was conducted in Organic Chemistry post graduate laboratory, Natural Science College, Jimma University. The purchased seeds were winnowed, washed, shade dried and ground into coarse powder using mortar and pestle. The coarse powder was weighed and then packed well in a clean plastic container to avoid the entrance of air and other surrounding material until extracted.

4.9. Preliminary Phytochemical Screening

The Preliminary phytochemical screening was conducted in Organic Chemistry Post Graduate Laboratory, Natural Science College, Jimma University. Different organic solvent extracts of *Lepidium sativum L.* seed were used to screen the following phytochemicals like alkaloids, phenolic compounds, flavonoids, saponins, steroids, terpenoids, and quinones.

The extract of the (80%) methanol, chloroform, hexane and acetone was prepared by dissolving the 15g seed powder in 50ml beaker separately for each solvent. The methods of screening employed were those described by (78,79) for the presence of various active components.

4.9.1. Test for Alkaloids (Wagner's reagent test)

One (1) ml of seed extract was treated with three (3) drops of Wagner's reagent (1.27g iodine and 2g potassium iodide in 100ml of water) and formation of a brown reddish precipitate indicates the presence of alkaloids

4.9.2. Test for Flavonoids (Alkaline reagent test)

Two (2) ml of seed extract was treated separately with 20% NaOH solution, the formation of intense yellow color that became colorless on the addition of dilute hydrochloric acid indicates the presence of the flavonoid.

4.9.3. Test for Phenolic compounds (Ferric chloride test)

Two (2) ml of seed extract was treated with aqueous 5% ferric chloride and the deep blue color of the solution shows the presence of phenol.

4.9.4. Test for Saponins (Foam test)

Two (2) ml of seed extract was added separately to 5 ml of water for two minutes; the mixture was mixed vigorously and left for three minutes. The formation of frothing indicates the presence of saponins.

4.9.5. Test for Steroid (Liebermann-burchard test)

One (1) ml of seed extracts was treated with chloroform, acetic anhydride and concentrated sulfuric acid and then, observed for the formation of dark pink or red color.

4.9.6. Test for Terpenoid (Salkowki test)

One (1) ml of chloroform was added to 2ml of extract followed by the addition of concentrated sulfuric acid. The appearance of a reddish brown precipitate color indicates the presence of terpenoid.

4.9.7. Test for Quinone

Addition of 2ml seed extract with 5 ml hydrochloric acid result in yellow colored precipitate indicates the presence of quinone.

4.10. Preparation of Plant Material Extract

The seed coarse powder (300g) was extracted by maceration in 80% methanol for 72 hours (three days) at room temperature by shaking three times per day throughout the maceration time. The mixture was first filtered using cotton wool and then with Whatman No. 1 filters paper. The residue was re-macerated for another 72h twice and filtered. The combined filtrate was separated by rotary evaporator at 50℃ and 90 rpm. Then, the filtrate was taken to the thermostatic oven at 40℃ and kept overnight to evaporate the remaining methanol. This was then lyophilized repeatedly using a freeze dryer (lyophilizer) at temperature of -40 ℃ and pressure of $133\text{x}10^{-3}$mbar until the water was completely removed and finally the obtained dried extract was weighed to determine percentage yield. Then, the total dried extract was harvested and kept in a desiccator to maintain dryness until used for the experiment (80,81).

$$\%\ \text{yield} = \frac{\textbf{weight of the dried extract}}{\textbf{weight of the seed powder}} \times \mathbf{100} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

4.11. Preparation of Potato and Deep Fried Palm Oil

4.11.1. Preparation of Potato

The potato was purchased from the market, Jimma, South Western Ethiopia. Potato was prepared by principal investigator in collaboration with street food vendors. Potato was washed, peeled and cut into slices of uniform size using a vegetable slicer. The sliced potatoes were kept in water, blotted with tissue paper and then, 500g was weighed for the frying process.

4.11.2. Preparation of Deep Fried Palm Oil

The preparation of deep fried palm oil was carried out according to the previously described methods of (16,33) with minor modification based on information gathered from street food vendors in Jimma. Palm oil was purchased from the market, Jimma, Ethiopia. Then, 2.5 L of fresh palm oil was added into a deep fat fryer and frying was carried out at a temperature of 200°C. A batch of 500g raw sliced potatoes was fried for 20 min and then, the fried potatoes batch was removed from the fryer. Then, the frying operation was carried out for a new potato batch. The frying procedure was done once daily for 5 consecutive days. The same oil was used repeatedly to fry the next batch of potatoes without adding any fresh palm oil to top up the lost oil during frying process. At the end of the frying, oil was taken out, filtered, kept in a bottle until used for animal diet preparation.

4.12. Animal Diet Preparation

Animal diet preparation was conducted in Veterinary Medicine postgraduate laboratory, College of Agriculture and Veterinary Medicine, Jimma University. The animal diet was prepared by mixing deep fried palm oil with normal mice pellet to contain 15% deep fried palm oil. The normal mice pellet diet of 85% w/w was mixed manually with prepared deep fried palm oil of 15% w/w. The mixtures were left to absorb the fried oils at room temperature overnight before the feeding was conducted (5,82).

4.13. Data Collection

The body weight of the mice was taken at the interval of a week to observe body weight change in all groups of animals during the study period. At the end of the study period, mice in all groups were fasted overnight and anesthetized with diethyl ether anesthesia, which was soaked in cotton wool and enclosed in a jar. Then, 2-2.5 ml of the blood was taken from each mouse through cardiac puncture and sacrificed by cervical dislocation. Then, the blood was collected with serum separator tube (SST) and left for 30 minutes at room temperature to clot. The serum was separated through centrifugation with a speed of 3000 revolutions per minute at room temperature for 10 minutes, then, it was pipetted off using a micropipette and transferred into another clean tube. Finally, the serum tube was put in the refrigerator until it was analyzed for lipid profiles and liver function tests.

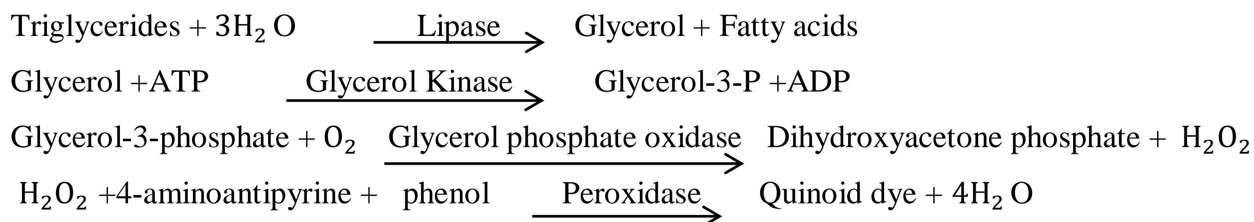
4.14. Serum Lipid Profiles

The lipid profiles: TC, TG, LDL and HDL were determined using SD Lipido Care ® analyzer following the manufacturer procedures.

4.14.1 Serum Triglyceride

Principle: Triglyceride (TG) is measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol and free fatty acids by the lipase enzyme. The glycerol is then phosphorylated by ATP to glycerol-3-phosphate and ADP in a reaction catalyzed by glycerol kinase. The glycerol-3-phophate is oxidized by glycerol phosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Then, Peroxidase catalyzes the redox-coupled reactions of H_2O_2 with 4-aminoantipyrine (4-AAP), producing a bright purple color (83).

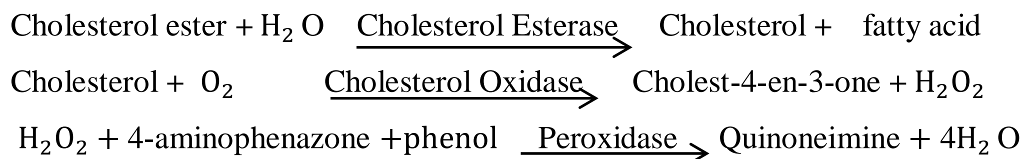
The reaction sequence is as follows:



4.14.2. Serum Total Cholesterol

Principle: Total cholesterol is measured enzymatically in serum in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. 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 (83).

The reaction sequence is as follows:

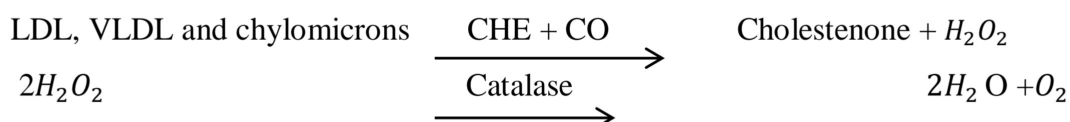


4.14.3. Serum High Density Lipoprotein

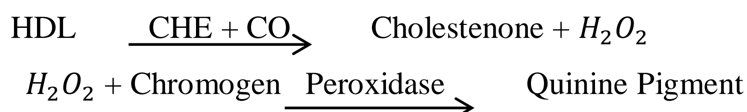
Principle: The HDL liquicolor test is a direct homogeneous enzymatic assay for the quantitative determination of HDL. The test combines two steps, the first step eliminating chylomicrons, VLDL and LDL by specific enzymatic degradation. The next step determines the remaining HDL by the well-known enzymatic reaction cascade cholesterol esterase (CHE), 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 (83).

Reaction principles are as follows:

First Step



Second step



4.14.4. Serum Low Density Lipoprotein

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL. The LDL was calculated using the empirical formula of Friedewald equation:

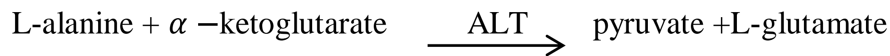
LDL-c = (Total cholesterol) $-\frac{(\text{TG})}{5}-$ (HDL), where (TG]/5 is an estimate of VLDL and all values were expressed in mg/dl. The Friedewald equation is not valid for calculating LDL if the serum TG is above 400 mg/dl (83).

4.15. Liver Function Tests

The liver function tests such as ALT, AST, ALP, Total bilirubin and Albumin were determined using fully automated ABX 400 Pentra clinical chemistry analyzer (China).

4.15.1. Alanine Aminotransferase

Alanine aminotransferase (ALT) is an enzyme involved in the metabolism of the amino acid alanine. ALT present in the sample catalyzes the transfer of the amino group from L-alanine to α-ketoglutarate forming pyruvate and L-glutamate. Pyruvate in the presence of NADH and lactate dehydrogenase (LDH) is reduced to L-lactate. In addition, NADH is oxidized to NAD+. The rate of decrease in absorbance of the reaction mixture at 340nm, due to the oxidation of NADH is directly proportional to the ALT activity (84).



4.15.2. Aspartate Aminotransferase

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to α-ketoglutarate to yield oxaloacetate and L-glutamate. Malate dehydrogenase (MDH) catalyzes the reduction of oxaloacetate with simultaneous oxidation of NADH to NAD^+.

AST is measured by the reagent rate analysis the coupled reaction with malate dehydrogenase (MDH) to reduce NADH (measured at a wavelength of 340nm) to NAD+. The rate of decrease in absorbance at 340 nm due to NADH depletion is proportional to the AST activity in the sample (84).

4.15.3. Alkaline Phosphatase

Alkaline phosphatase (ALP) measurements are most useful in diagnosing or monitoring hepatobiliary diseases, particularly extrahepatic obstruction due to stones or pancreatic cancer causing cholestasis, and bone diseases associated with increased osteoclastic activity. ALP is measured by the reagent rate analysis of p-nitrophenylphosphate, with cofactors zinc and magnesium, to form p-nitrophenol. The rate of increase of p-nitrophenol formation, measured at 405 nm is proportional to the activity of alkaline phosphatase in the sample (84).

p- nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ p- nitrophenylphosphate + phosphate

4.15.4. Albumin

Albumin is measured as an end point chemical reaction with albumin binding to bromocresol green (BCG), which is an anionic dye, in an acidic environment. The increase in absorbance at 620 nm of the green colored product is proportional to the albumin concentration in the sample (85).

4.15.5. Total Bilirubin

Total bilirubin is measured as an end point chemical reaction using diazotization to produce azobilirubin. Increases in absorbance generated by blue colored azobilirubin and measured at 546 nm is proportional to the concentration of total bilirubin in the sample (86).

4.16. Liver Histopathology

A mouse of each group was sacrificed following diethyl ether anesthesia. Mice were laid on a clean paper and then, a vertical midline incision with scissors cut from the neck to pubis and opens the peritoneum. Then, the whole liver tissue was taken and transferred to organ tube containing the 10% formalin for preservation and transportation. Then, in gross room liver tissue was excised from specific part of liver lobe by pathologist and transferred into tissue processing cassette. The tissue was processed overnight in open tissue processor.

The liver tissue in the cassette rotates overnight over different reagent containing jars starting from the 10% formalin which completely immerses the tissues for the purpose of fixation. Then, tissues were dehydrated in a series of an increased ordered ethanol concentration of (70%, 80%, 90% and 100%). Xylene was used to remove ethanol from the tissue and replace it with fluid which is soluble with paraffin (87).

The tissues were embedded in paraffin wax with the help of electro-thermal wax dispenser to form tissue blocks in squared metallic plates block moulds. The blocks were then labeled, and placed in a refrigerator until sectioned. Microtome was used for sectioning of tissue blocks manually. The paraffin block having tissue was put in the rotary microtome. The ribbon of sections was carefully picked from the knife by a blunt forceps to float in a water bath of 40℃ to remove folds in the sections. Unfolded sections were picked by clean microscope glass slides and were placed in an oven maintained at a temperature of 56℃ for 15 minutes for proper drying and better adhesion. The tissue sections were then cooled, dried and stained (88).

The paraffin wax was removed from the tissue sections using xylene. The sections were then immersed in a series of descending alcohol concentration (100, 95, 70, and 40%) to remove xylene after which distilled water was used to hydrate the tissue. The hydrated sections were immersed in hematoxylin for 3-5 minutes with an eosin counterstained and agitated with acid alcohol to prevent over staining. Sections were immersed in a mixture of sodium 30 bicarbonate, ethanol and distilled water to give blue colour to the nucleus. Then it was immersed in 95% alcohol and eosin to give pink color to the cytoplasm. Finally, tissue sections were dehydrated in 95% alcohol, cleared in xylene and mounted by adding a drop of DPX (Dibutyl Phthalate in Xylene) mounting medium on the section to cover the microscopic glass with cover glass and to increase the refractive index of the tissue under light microscope (89).

4.17. Study Variables

4.17.1. Dependent Variables

- Serum lipid profile
- Serum liver function tests
- Liver histopathology

4.17.2. Independent Variables

- *Lepidium sativum L.* seed extract

4.18. Operational Definitions

Normal control – group that fed on normal mice pellet + distilled water

Negative control – group that fed on deep fried palm oil diet + distilled water

Treatment group- is a group that fed on deep fried palm oil diet + *Lepidium sativum* seed extract at dose of 200 and 400 mg/kg/day

***Lepidium sativum* seed**-are reddish brown and two per pod of the plant (61).

***Lepidium sativum* seed extract-** is extract that prepared by using hydromethanol as a solvent

Lipid profile- is a panel of blood tests for measurement of lipids in serum which include TC, TG, HDL, and LDL (90).

Deep fried palm oil- palm oil that repeatedly deep fried daily once for 20min in five consecutive days at 200℃

Deep fried palm oil diet = normal mice pellet that contain (15% w/w) deep fried palm oil

Liver function tests is a panel of blood tests for measurement of serum liver damage markers that indicate damage to liver cells and most commonly include ALT, AST, ALP, total bilirubin and albumin (45).

Liver Enzymes: include the most commonly used liver damage markers such as ALT, AST, and ALP

4.19. Data Analysis

The data were entered to the epi-data version 3.1 and exported to statistical package for social science (SPSS) version 25 for analysis after it was checked and cleaned. It was expressed as mean ± SD. One-way ANOVA was done to determine statistical differences among all groups of the study. This was followed by Tukey post hoc test using SPSS software version 25 and ($p < 0.05$) considered as statistically significant. The results were presented by tables and figures. The qualitative microscopic examination of the photomicrography of liver section was performed by trained pathologists.

4.20. Data Quality Management

All chemicals and reagents were checked for their expiry date. Equipment's of analytical grade and properly functioning one was used. The fresh animal diet and the seed extract dose were prepared and administered daily to the experimental animals. The sample was collected, transported and stored according to the standard guideline until used. Each biochemical test was performed by experienced laboratory technologists following standard operational procedures. The chemistry analyzer was checked by using controls to confirm either performing the test correctly or not. The liver histopathology investigation was performed by the trained and experienced senior pathologists. The pathologists were blinded to prevent the bias among the group of mice. The histopathology investigation was performed twice by pathologists to reassure the consistency of the findings.

4.21. Ethical Considerations

The research was conducted after getting an ethical approval letter from the Jimma University Institutional Review Board with a reference No. of IHRPGD/714/2020 and the support letter was written to Tropical and Infectious Disease Research Center, Chemistry, Veterinary Medicine, and Pathology Department from Biomedical Science Department. All experimental activities were carried out following the ethics of experimental animal which comply with scientific and ethical guidelines. For the prevention of COVID 19 wearing of face mask, maintaining physical distance and washing of our hand with detergent every time especially while entering the experimental lab class in every activity during the study period was performed seriously.

4.22. Dissemination Plan

The research document that contains the study results will be submitted to biomedical science department; Research and Post Graduate programs coordinating office of Jimma University as partial fulfillment of a master's degree in Medical Biochemistry. The research will also be disseminated widely through publishing on a reputable journal. Besides, the research finding will also be disseminated through presenting in different training and workshop.

5. RESULTS

5.1. Preliminary Phytochemical Screening

The preliminary phytochemical screening of *Lepidium sativum L.* seed was revealed the status of phytochemical constituents such as alkaloid, flavonoid, phenol, steroid, saponin, and quinone using different organic solvent extract as shown in (Table 2).

Table 2 : Preliminary phytochemical screening of *Lipidium sativum L.* seed

Phytochemical Constituents	Status			
	HME	HE	CE	AE
Alkaloid	+	+	+	+
Terpenoid	+	−	+	+
Flavonoid	+	−	−	+
Phenol	+	−	−	−
Steroid	+	−	−	−
Saponin	+	−	−	−
Quinone	+	−	+	+

The letter Indicate: HME-Hydromethanolic extract, HE-Hexane extract, CE-chloroform extract, AE-Acetone extract. $(-)$ = absent, $(+)$ = present

5.2. Percentage Yield of *Lepidium sativum L.* Seed Extract

The amount of crude extracts (hydromethanol extract) which was obtained from 300g coarse powder of *Lepidium sativum L.* seed was 37.92g. Therefore, the percentage yield was calculated and given as:

$$\% \text{ yield} = \frac{\mathbf{37.92}}{\mathbf{300}} \times \mathbf{100} = 12.6\% \text{ (w/w)} \quad (2)$$

5.3. Effect of *Lepidium sativum L.* Seed Extract on Body Weight

As shown in the figure 4, Group II showed non-significant ($p > 0.05$) decrement in body weight at the end of day 7 but started to increase in the remaining day like 14, 21 and 28 day when compared to normal control Group I. Group III and IV was showed non-significant ($p > 0.05$) decrement in body weight in day 14, 21 and 28 when compared to Group II.

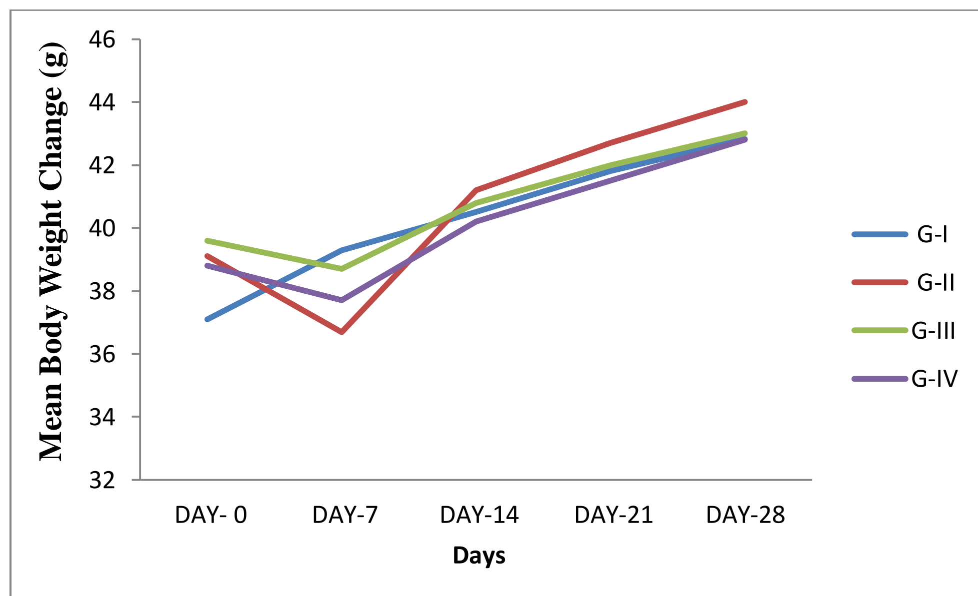


Figure 4 : Effect of *Lepidium sativum L.* Seed Extract on the body weight at different day

In the present study, the body weight change of the Group II (4.83 ± 2.48) that fed on the deep fried palm oil diet decreased non-significantly (P> 0.05) as compared to the Group I normal control (6.33 ± 4.76). The body weight change of the Group III and IV (3.33 ± 1.21, 4.00 ± 2.28), respectively decreased non-significantly (p> 0.05) as compared to the Group II (4.83 ± 2.48) that fed on the deep fried palm oil diet.

Table 3: Comparison of Mean ± SEM value of body weight among the four group**s**

Groups	Body weight (g)		
	Day 0	Day 28	Change
I	37.10 ± 1.93	42.83 ± 0.60	6.33 ± 1.94
II	39.10 ± 0.90	44.00 ± 0.36	4.83 ± 1.01
III	39.60 ± 0.76	43.00 ± 0.63	3.33 ± 0.49
IV	38.80 ± 0.79	42.80 ± 0.30	4.00 ± 0.93

5.4. Effects of *Lepidium sativum L.* Seed Extract on Lipid Profile

As shown in Table 4, the group II serum TC value (134.83 ± 3.53) increased significantly ($P < 0.05$) when compared with group I normal control serum TC value (112.66 ± 3.20). The group III serum TC value (123.00 ± 3.86) showed non-significant ($P > 0.05$) decrement when compared to group II serum TC value (134.83 ± 3.53). The group IV serum TC value (116.66 ± 1.28) decreased significantly ($P < 0.05$) when compared to group II serum TC value (134.83 ± 3.53).

The group II serum HDL value (65.33 ± 1.47) decreased significantly ($P < 0.05$) when compared to group I normal control serum HDL value (74.00 ± 0.77). The group III serum HDL value (67.50 ± 1.43) showed non-significant ($P > 0.05$) increment when compared to group II serum HDL value (65.33 ± 1.47). The group IV serum HDL value (76.83 ± 1.53) was increased significantly ($P < 0.05$) when compared to group II serum HDL value as shown in Table 4.

As shown in (Table 4), the group II serum LDL value (29.17 ± 4.98) increased significantly ($P < 0.05$) when compared to group I normal control serum LDL value (13.50 ± 2.24). The group III serum LDL value (25.33 ± 5.05) showed non-significant ($P > 0.05$) decrement when compared to group II serum LDL value (29.17 ± 4.98). The group IV serum LDL value (12.66 ± 2.41) decreased significantly ($P < 0.05$) when compared to group II serum LDL value (29.17 ± 4.98).

The group II serum TG value (202.00 ± 5.05) increased significantly ($P < 0.05$) when compared to group I normal control serum TG value (126.17 ± 4.98). The group III and IV serum TG values (151.33 ± 3.44, 140.00 ± 2.54), respectively, decreased significantly ($P < 0.05$) when compared to group II serum TG value (202.00 ± 5.05).

Table 4 : Comparison of the Mean ± SEM value of Lipid profile among the four groups of the male Swiss albino mice

Groups	Serum lipid level (mg/dl)			
	TC	HDL	LDL	TG
I	112.66 ± 3.20	74.00 ± 0.77	13.50 ± 2.24	126.17 ± 4.98
II	134.83 ± 3.53^{a}	65.33 ± 1.47^{a}	29.17 ± 4.98^{a}	202.00 ± 5.05^{a}
III	123.00 ± 3.86	67.50 ± 1.43^{a}	25.33 ± 5.05	151.33 ± 3.44^{ab}
IV	116.66 ± 1.28^{b}	76.83 ± 1.53^{b}	12.66 ± 2.41^{b}	140.00 ± 2.54^{ab}

The results were expressed as mean ± SEM, P < 0.05. Symbols represent statistical significance. a =considered significantly different at P < 0.05 when compared with the normal control group. b = considered significantly different at P < 0.05 when compared with the negative control group.

5.5. Effects of *Lepidium sativum L.* Seed Extract on Liver Enzymes

The group II serum ALT level (65.66 ± 1.11) increased significantly (P < 0.05) when compared to group I normal control serum ALT level (38.33 ± 1.89). The group III serum ALT level (58.83 ± 3.05) showed non-significant (P > 0.05) decrement when compared to group II serum ALT level (65.66 ± 1.11). The group IV serum ALT level (41.1 ± 2.24) decreased significantly (P < 0.05) when compared to group II serum ALT level (65.66 ± 1.11) as shown in Table 5.

As shown in Table 5, the group II serum AST level (131.00 ± 5.11) increased significantly (P < 0.05) when compared to group I normal control serum AST level (62.83 ± 2.48). The group III and IV serum AST levels ($94.8 \pm 5.12,\ 86.33 \pm 0.71$), respectively, decreased significantly (P < 0.05) when compared to group II serum AST level (131.00 ± 5.11).

As shown in Table 5, the group II serum ALP level (173.00 ± 3.46) increased significantly (P < 0.05) when compared to group I serum ALP level (91.33 ± 1.64). The group III and IV serum ALP levels ($160.83 \pm 2.7, 154.00 \pm 1.65$), respectively, showed significant (P < 0.05) decrement, when compared to group II serum ALP level (173.00 ± 3.46).

Table 5 : Comparison of the Mean ± SEM value of Liver Enzymes among the four groups of male Swiss albino mice

Groups	Liver Enzymes		
	ALT (U/L)	AST (U/L)	ALP (U/L)
I	38.33 ± 1.89	62.83 ± 2.48	91.33 ± 1.64
II	65.66 ± 1.11^{a}	131.00 ± 5.11^{a}	173.00 ± 3.46^{a}
III	58.83 ± 3.05^{a}	94.8 ± 5.12^{ab}	160.83 ± 2.7^{ab}
IV	41.1 ± 2.24^{b}	86.33 ± 0.71^{ab}	154.00 ± 1.65^{ab}

The results were expressed as mean ± SEM. Symbols represent statistical significance. a =considered significantly different at P < 0.05 when compared with the normal control group. b = considered significantly different at P < 0.05 when compared with the negative control group.

5.6. Effects of *Lepidium sativum L.* Seed Extract on Albumin and Total bilirubin

The group II serum albumin level (2.22 ± 0.11) decreased significantly (P < 0.05) when compared to group I normal control serum albumin level (3.62 ± 0.18). The group III serum albumin level (2.95 ± 0.29) showed non-significant (P > 0.05) increment when compared to group II serum albumin level (2.22 ± 0.11). The group IV serum albumin levels (3.07 ± 0.13) were significantly (P < 0.05) increased when compared to group II serum albumin level (2.22 ± 0.11) as shown in Table 6.

The group II serum total bilirubin level (0.48 ± 0.07) showed significant (P < 0.05) increment when compared to the group I serum total bilirubin level (0.17 ± 0.01). The group IV serum total bilirubin levels (0.23 ± 0.38) shown significant (P < 0.05) decrement when compared to group II serum total bilirubin level (0.48 ± 0.07).

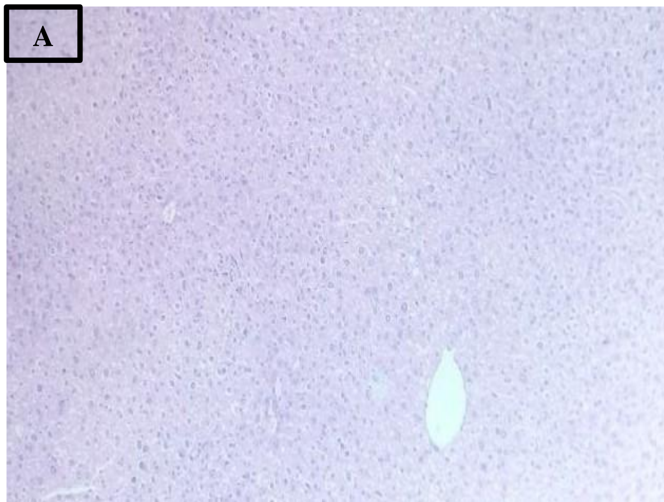
Table 6 : Comparison of the Mean ± SEM value of Albumin and Total bilirubin among the four groups of male Swiss albino mice

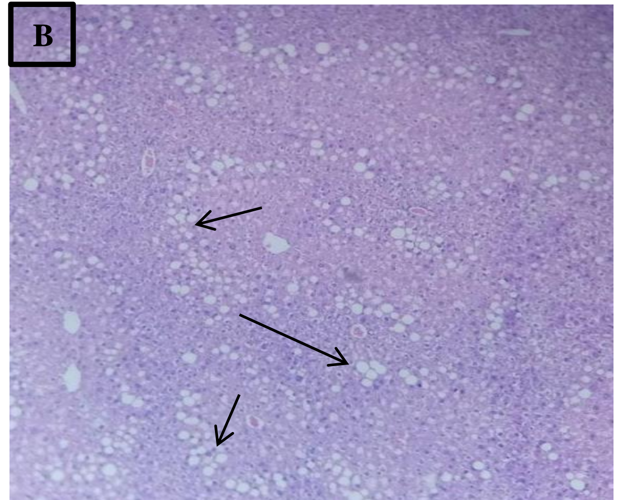
Groups	Albumin (mg/dl)	Total bilirubin (mg/dl)
I	3.62 ± 0.18	0.17 ± 0.01
II	2.22 ± 0.11^a	0.48 ± 0.07^a
III	2.95 ± 0.29	0.38 ± 0.04^a
IV	3.07 ± 0.13^b	0.23 ± 0.38^b

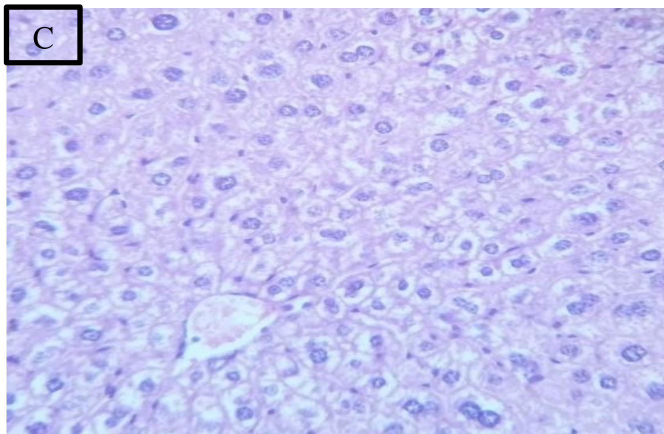
The results were expressed as mean ± SEM. Symbols represent statistical significance. a =considered significantly different at P < 0.05 when compared with the normal control group. b = considered significantly different at P < 0.05 when compared with the negative control group.

5.7. Liver Histopathology

Histopathological examination of the liver of control group revealed normal hepatic parenchyma composed plates of hepatocytes, central veins, and hepatic vessels (Fig.5; A). Histopathological alterations were observed in the deep fried palm oil diet fed group II included hepatic parenchyma with sever vacuolar and fatty change (Fig.5; B). Liver sections of mice treated with deep fried palm oil diet + 200mg/kg/day of LSSE showed the hepatocyte with mild vacuolar degeneration (Fig.5; C). Livers of section of mice treated with deep fried palm oil diet + 400mg/kg/day of LSSE appeared more or less similar to the normal control group liver sections (Fig.5; D).







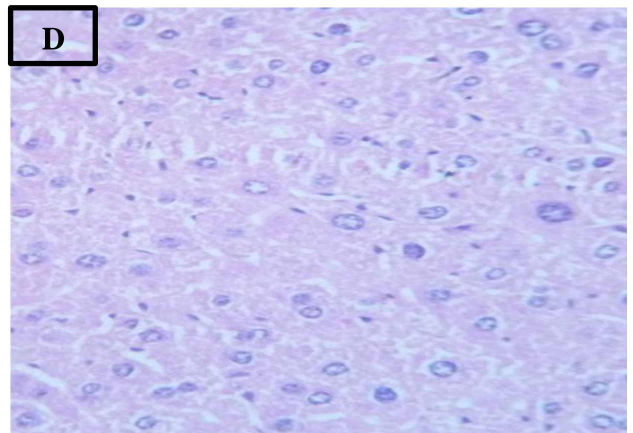


Figure 5: Photomicrographs of 10×, 40×, H and E stained liver sections of mice. (A) Group I, (B) Group II -Liver sections present severe degenerative changes in hepatocytes including fatty changes (arrows), (C) Group III, (D) Group IV.

6. DISCUSSION

Medicinal plants are getting great attention as valuable sources of food additives and bioactive substances with beneficial health effects. The incorporation of the products of medicinal plants in the daily food intake may be one of the highly used methods to modify the sensory characteristics of the diet and this in turn also resolve the problems of underutilization of medicinal plants. Previous studies reported, intake of food which is rich in plant bioactive compounds such as polyphenols, flavonoids, saponin and others, in particular, may exert beneficial effects towards human health (91) .

6.1. Effects of *Lepidium sativum L.* Seed Extract on Lipid Profile

In the present study, the group II serum TC value increased significantly ($P < 0.05$) when compared to group I serum TC value. This study finding was in line with the previous study finding of Famurewa *et al* (33) which showed the repeatedly heated palm oil induces increment in TC value. The increase in the serum TC in the present study might be due to the increased saturated fatty acid content in the palm oil (7).The group IV that administered LSSE at 400 mg/kg/day serum TC value decreased significantly ($P < 0.05$) when compared to group II serum TC value that fed on deep fried palm oil diet. This study finding was in harmony with the study results of Bushra *et al* (93) that revealed *Lepidium sativum* seed effect in reducing TC in high fat diet fed rats. This might be attributed due to flavonoid (naringenin) inhibition of cholesterol biosynthesis through inhibition of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme which mediates the major step in cholesterol biosynthesis (93).

Besides, it might be also due to the saponin constituens of *Lepidium sativum* seed that form insoluble saponin cholesterol complexes within gastrointestinal tract which prevents absorption of cholesterol like saponin of the *Thymus vulgaris* (90). However, the present study findings contradict with Hanan *et al* (94) study report that showed the elevation of serum TC in rats fed diet supplemented with *Lepidium sativum* seed. The exact causes for these variations are unclear. However, it may be due to the variations in the mode of *Lepidium sativum* seed extract administration, plant variety (geographical differences) or extraction method differences.

In the current study, the group II serum HDL value was decreased significantly ($P < 0.05$) when compared to group I serum HDL value. This study finding was in agreement with the previous study findings report of Falade *et al* (49) that showed a decrement in HDL in thermally oxidized palm oil diet fed rats. The decrease in HDL levels in the present study might be due to intake of the increased trans-fat concentration (26). In contrast, this study finding was inconsistent with the study findings of Hussein that showed an increment in HDL on mice that fed reused palm oil (95). The exact causes for variations are not clear. However, the variation might be due to the difference in the temperature of frying, the frying methods and the types of animals used. In the present study, the group IV that administered the LSSE at the dose of 400 mg/kg/day serum HDL value was showed significant ($p< 0.05$) increment when compared to group II negative control serum HDL value. The present study result was consistent with the study findings of Shukla *et al* (96) that revealed the *Lepidium sativum L.* seed extract increasing effect on HDL in high cholesterol diet fed rats. This might be due to the better efficiency of flavonoid constituents (apigenin) of the extract towards increasing HDL. The flavonoid (apigenin) constituents enhance the expression of the ABCA1 gene and this facilitate the cholesterol efflux from the macrophage and inturn increase the HDL in the circulation (97).

In the present study, the group II serum LDL value increased significantly ($p< 0.05$) when compared to group I serum LDL value. These study findings were in line with study findings of Hassan *et al* (7) that justified the increment in LDL in rat that fed repeatedly fried palm oil. Oxidation reaction during deep frying causes changes in fatty acid configuration from the cis isomer to the *trans* isomer. The increase in LDL levels in the present study might be due to intake of the increased trans-fat concentration (26).

The group IV that administered LSSE at 400 mg/kg/day serum LDL value decreased significantly ($p< 0.05$) when compared to group II serum LDL value. The present study result was in harmony with the study finding of Sharma *et al* (98) that revealed the LDL lowering effect of *Lepidium sativum L.* seed in both high fat diet fed and diabetic induced mice. The possible mechanism for the decreased LDL could be due to the flavonoid (kaempferol) constituents of seed on inducing the increased low-density lipoprotein receptor gene expression (99).

The present study findings were inconsistent with, Thnaian (100) study report that showed the elevation of serum LDL level in rats fed basal diet mixed with cholesterol and *Lepidium sativum* seed powder. The exact causes for these variations are unclear. However, it may be due to the variations in the mode of *Lepidium sativum* seed administration and low concentration of the administered dose.

In the present study, the group II serum TG value showed a significant ($p < 0.05$) increase when compared with the group I normal control serum TG value. This study finding was in agreement with the previous study result of Okwari *et al* (101) that revealed increased triglycerides level in thermoxidized palm oil diet fed Rat. The increase in triglyceride level in negative control group after deep fried palm oil diet fed could be due to the increase in the availability of substrate free fatty acids for esterification (102). However, this study finding was inconsistent with the study findings of (7,49). The exact reasons for variations are not clear. However, the variation might be due to the difference in the process of frying, and the types of animals used. The TG of group III and IV that administered LSSE at the dose of 200 and 400 mg/kg/day, respectively were significantly ($p < 0.05$) decreased when compared to group II. This study finding was in harmony with the study result of Alharbi *et al* (94) in which *Lepidium sativum L.* seed showed a decrement in triglycerides level in diabetic Rat. These might be due to *Lepidium sativum L.* seed secondary metabolites inhibition of absorption and enhanced excretion of lipids.

6.2. Effects of *Lepidium sativum L.* Seed Extract on Liver Enzymes

The serum ALT, AST, and ALP are among serum liver function tests , with their increase in the serum indicating liver damage (103). In the present study, the group II serum ALT level were increased significantly ($P<0.05$) when compared to group I serum ALT level. The present study finding was in agreement with the study result of Jaarin *et al* (104) on repeatedly heated palm oil fed experimental rats.

The probable explanation might be due to lipid peroxidation that produce a breakdown and loss of permeability of the cellular membrane (46). In the current study, the group IV that administered LSSE at 400mg/kg/day showed a significant ($P < 0.05$) decrease in serum ALT level when compared to group II serum ALT level. The result of the present study was in line with study finding of Fawiziah *et al* (94) on diabetic rats fed on basal diets mixed with *Lepidium sativum* seed. This improvement of the liver ALT might be attributed due to the presence of naringin flavonoid in the seed extract (73).

In the present study, the group II serum AST levels increased significantly ($P< 0.05$) when compared to group I serum AST levels. The present study finding was in agreement with the study result of Hussein *et al* (95) on reused palm oil fed albino mice. This might be because of the damaged structural integrity of the liver resulted in the rupture of the plasma membranes and their release into blood circulation (90). In the current study, the LSSE at 200 and 400 mg/kg/day showed a significant ($P < 0.05$) decrease in serum AST level when compared to group II serum AST level. The result of the present study was in line with the study finding of Mamdoh *et al* (105) on methanolic extract of *Lepidium sativum* seeds on mice infected with Trypanosoma evansi. The *Lepidium sativum* seed extract contains important flavonoids such as naringenin, naringin, kampferol, apigenin, and luteolin. These compounds have antioxidant and antiradical capacity that protect cell membrane from radical damage. The decreased serum AST level in the present study might be due to radical scavenging activity of flavonoids that prevent lipid peroxidation and normalize the lipid profile of hepatocyte membrane (69,106).

In the present study, the group II serum ALP levels increased significantly ($P< 0.05$) when compared to group I serum ALP levels. The present study finding was in agreement with the study result of Amany *et al* (90) on thermally oxidized oil fed rabbits. The increase in ALP might be due to intrahepatic, extrahepatic biliary obstruction of the liver (94). In the current study, the LSSE at 200 and 400 mg/kg/day showed a significant ($P < 0.05$) decrease in serum ALP level when compared to group II serum ALP level. This study result agrees with the result of the Zamzami *et al* (107) on the effect of *Lepidium sativum* seed on hepatotoxicity induced rabbits. This might be due to high polyphenol content in the seed of the *Lepidium sativum* that protect the liver from the damage induced by oxidants (66).

6.3. Effects of *Lepidium sativum L.* Seed Extract on Albumin and Total bilirubin

In the present study, the group II serum albumin level decreased significantly (P < 0.05) when compared to group I normal control serum albumin level. The present study finding was in agreement with the result of Adedayo *et al* (49) on thermally oxidized palm oil fed rats. The lowered serum albumin level in the present study might be due to decreased protein digestibility and absorption due to cross-linking reactions of secondary lipid oxidation products with proteins (26). In the present study, the group IV that administered LSSE at 400mg/kg/day,increased significantly (P < 0.05) in serum albumin level when compared to group II serum albumin level. The current study finding was consistent with the study result of Zamzami *et al* (107) on the Amelioration of *Lepidium sativum* seeds in CCl-4-induced hepatotoxicity in Rabbits. This might be due quercetin content of the seed that scavenge free radicals and bind transition metal ions (67).

The group II serum total bilirubin level showed significant (P < 0.05) increment when compared to the group I serum total bilirubin level. The present study finding was in agreement with the study result of Amany *et al* (90) on thermally oxidized oil fed rabbits. The elevated level of serum total bilirubin might be due its leakage from hepatocytes to plasma as a result of hepatic obstruction to bile outflow. The group IV that administered LSSE at 400mg/kg/day serum total bilirubin level shown significant (P < 0.05) decrement when compared to group II serum total bilirubin level. The current study finding was consistent with the study result of Zamzami *et al* (107) on the amelioration of *Lepidium sativum* seeds in CCl-4-induced hepatotoxicity in rabbits. These might be attributed due to the improvement of the liver functions to the presence of important flavonoids such as naringin and naringenin (69).

6.4. Liver Histopathology

Concerning histopathological evaluation in the present study, the male Swiss albino mice liver exposed to deep fried palm oil diets only showed hepatic parenchyma with severe vacuolar and fatty change. The result of the present study are consistent with the result of Ahmed et al (90) that reported severe degenerative changes in hepatocytes including fatty changes in which hepatocytes showing ring appearance in thermoxidized palm oil fed rabbits.

The present study revealed that concurrent treatment of male Swiss albino mice that administered deep fried palm oil diet with LSSE improved their liver histopathological architecture in the form of mild vacuolar degeneration (at 200 mg/kg/day of LSSE), normal hepatic parenchyma composed of central veins and portal tracts with portal veins (at 400 mg/kg/day of LSSE), thus approving ameliorative effect of LSSE. The present study results are in line with Zamzami (107) that reported the improvement of the histological structure in rabbits treated with LSSE in concurrent to CCl-4 administration.

7. LIMITATIONS OF THE STUDY

- Analysis of the products which formed during frying oils was not performed.
- Only two different doses of seed extract were investigated to assess the effect of the *Lepidium sativum L.* extract.
- Phytochemical analysis of the specific marker compound and their content of the *Lepidium sativum L.* extract was not performed.
- The comparison of *Lepidium sativum L.* seed extract with the standard drugs having lipid lowering and hepatoprotective effects was not conducted.

8. CONCLUSIONS AND RECOMMENDATIONS

8.1. Conclusions

The *Lepidium sativum L.* seed extract at 400 mg/kg/day showed more significant decreasing effect on TC, TG and LDL while significant increasing effect on HDL of male Swiss albino mice fed with deep fried palm oil diet as compared with the non-treated groups. This implies that seed extract has a prominent ameliorating effect on deep fried palm oil diet induced alteration of the lipid profile of male Swiss albino mice. Besides, the *Lepidium sativum L.* seed extract (at 400 mg/kg/day) showed more significant decreasing effect on ALT, AST, ALP, and total bilirubin while increasing effect on serum albumin level. This indicates the restoring capacity of *Lepidium sativum L.* seed extracts on the deranged liver function tests of male Swiss albino mice fed on deep fried palm oil diet. Moreover, the *Lepidium sativum L.* seed extract at 400 mg/kg/day restored the liver histopathology of mice almost similar towards the normal control group. Hence, it could be concluded that 400mg/kg/day doses of *Lepidium sativum L.* seed extract has a better effect on lipid profile, liver function tests and liver histopathology than 200mg/kg/day dose. Therefore, the *Lepidium sativum L.* seed extract can be helpful in preventing future damages caused by deep fried palm oil diet, such as alteration of lipid profile, that mainly result in cardiovascular diseases which is among the main causes of death and also the liver damage that inturn induces lipid profile alteration.

8.2. Recommendations

Based on this research, we recommend the following point:

- Awareness creation mostly to the street food vendors on the negative health effects associated with reusing the deep fried oil should be enhanced to restrict them from reusing the deep fried oil for food preparation.
- The effect of *Lepidium sativum L.* seed using a different form of solvent should be assessed on other biochemical parameters. As the current seed extract preliminary screening showed it contains important phytochemical constituents, especially by a solvent like acetone.
- The future study should focus on the antidyslepidemic and hepatoprotective effect of *Lepidium sativum* seed extract in comparison to the standard drugs.
- The future study should focus on the effect of *Lepidium sativum L.* root, leaf in addition to parts of the plant on different organ functions.

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ANNEXES

Annexes I: Plant Material Preparation

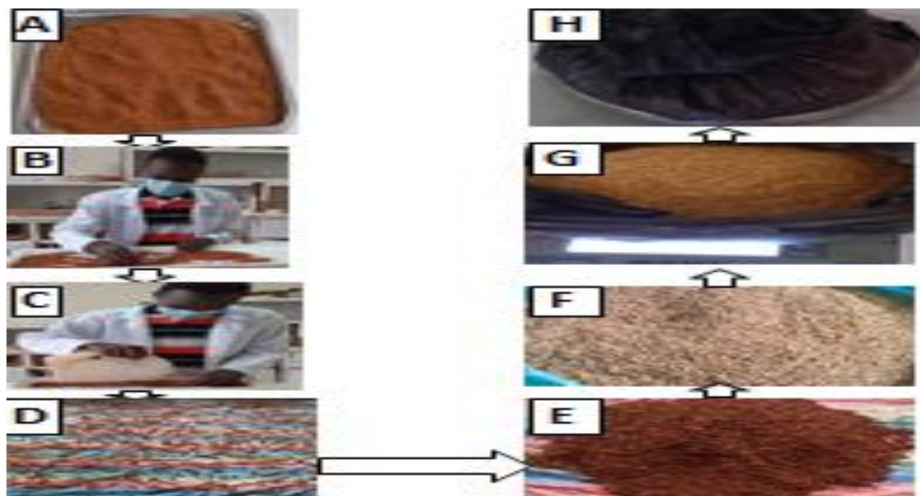

Figure 5 : Plant material preparation. The letter indicate: (A) Seed Purchased, (B) Winnowed, (C) Washed, (D) Drying, (E) Dried seed, (F) Powdered form, (G) Weighed, (H) kept in clean plastic

Annexes II: Preparation of plant material extract

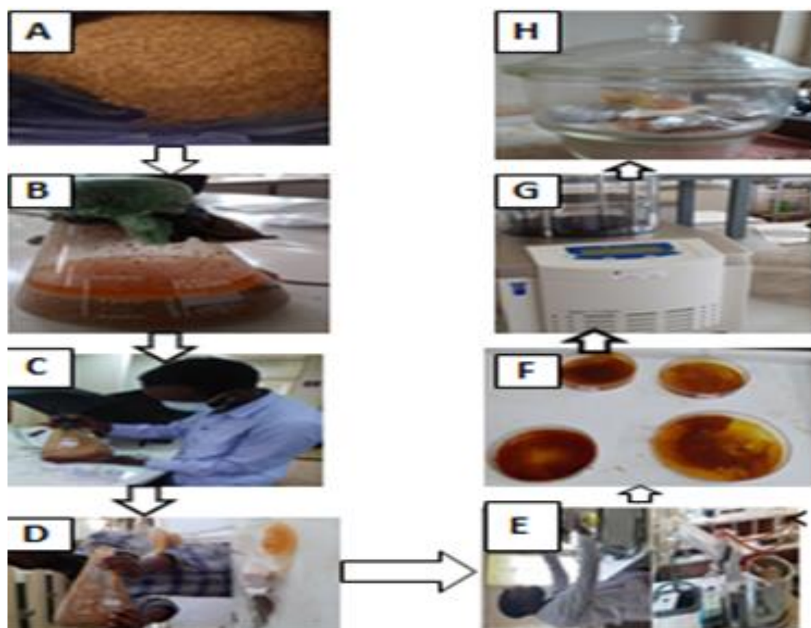

Figure 6 : Preparation of plant material extract (at Organic Chemistry Post Graduate Laboratory, by PI, in July 2020). The letter indicates: (A) weighed Powdered form, (B) Macerated, (C) Shaked,(D) Filtered, (E) Concentrated, (F) Separated into a different disc, (G) Freeze dried using lyophilizer, (H) Desiccator

Annexes III: Potato preparation

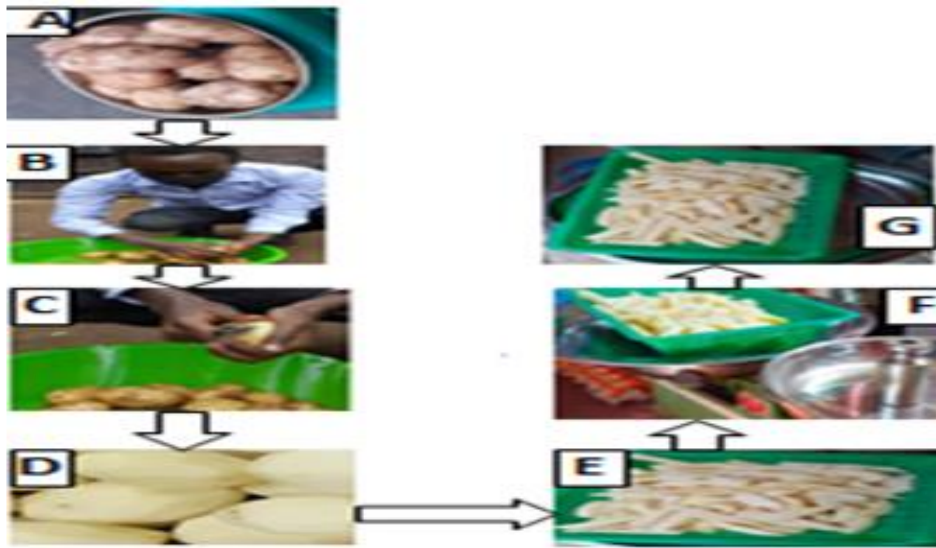

Figure 7 :Potato preparation (at street food vendor working shade, by collaboration with street food vendor and PI, in July 2020).The Letter indicates**:** (A) Purchased potato, (B) washed, (C) peeled, (D) peeled potato ,(E) sliced, (F) weighed

Annexes IV: Preparation of Deep Fried Palm Oil

Figure 8: Frying oil (at street food vendor working shade, in July 2020). The letter indicates: (A) Purchased palm oil, (B) Poured to deep fat fryer, (C) sliced potato (D) sliced potato added to fried palm oil, (E) frying (F) deep fried palm oil at the first day (G) deep fried palm oil at five days (H) kept in a bottle till use

Annexes V: Animal Diet Preparation

Figure 9 : Animal diet preparation (at veterinary medicine postgraduate laboratory, by PI, in July 28-August 27, 2020). The letter indicates: (1a) deep fried palm oil, (1b) normal mice pellet, (2a) weighing oil, (2b) weighing normal mice pellet, (3a) weighed oil , (3b) weighed normal mice pellet, (4) manually mixing oil and normal mice pellet, (5) deep fried palm oil diet

Annexes VI: Body Weight Follow Up Checklist

Table 7: Checklist for follow up of weight of mice per seven day

Group No.	Mice No.	Day 0	Day 7	Day 14	Day 21	Day 28
1	1					
	2					
	3					
	4					
	5					
	6					
2	1					
	2					
	3					
	4					
	5					
	6					
3	1					
	2					
	3					
	4					
	5					
	6					
4	1					
	2					
	3					
	4					
	5					
	6					

Annexes VII: Animals Grouping and Dose Administration

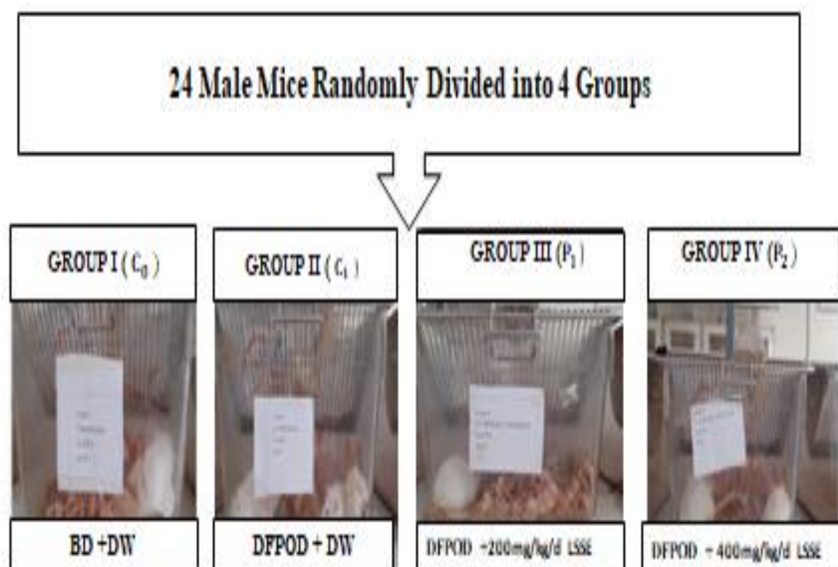

Figure 4.6: The experimental setup in the laboratory (at veterinary medicine postgraduate laboratory, by PI, in August 2020). The letter indicates: $\mathbf{C_0}$-Normal control, $\mathbf{C_1}$- Negative control, $\mathbf{P_1}$-Treatment one, $\mathbf{P_2}$- Treatment two

Annexes VIII: Preliminary Phytochemical Screening results

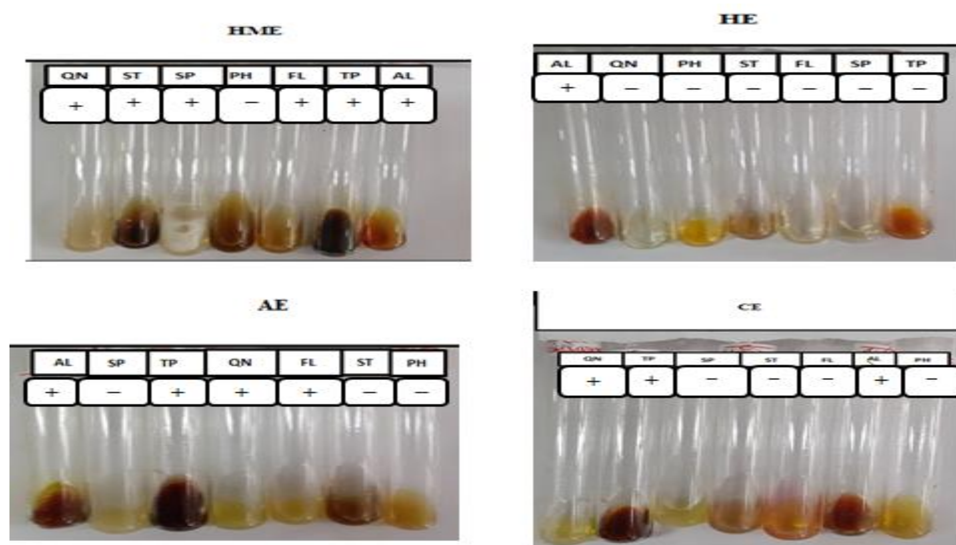

Figure 10: Preliminary Phytochemical Screening results. The letter indicates (**HME**-Hydromethanolic extract), (**HE**-Hexane extract), (**CE**-chloroform extract), (**AE**-Acetone extract). **AL**-alkaloid, **TP**-Terpenoid, **FL**-flavonoid, **Sp**- saponin, **PH**-phenol, **QN**-Quinone, and **ST**-Steroid. $(-)$ =Absence, $(+)$ =Presence.