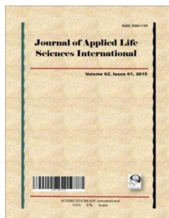

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Comparative Study on the Effects of Locally and Industrially Processed Olive Oil on Some Selected Organs of Albino Wister Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GI and OAG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WL and AJ managed the analyses of the study. Author GI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Olive oil is an important source of mono-unsaturated fat and a prime component of the Mediterranean diet. The beneficial health effects of olive oil are due to both its high content of mono-unsaturated fatty acids and its high content of anti-oxidative substances. This study is aimed at comparing the effects of industrially processed olive oil sold in Nigeria and locally processed olive oil in Plateau State Nigeria. Albino rats of weight 173 to 220 g were fed with normal feed in the University of Jos animal house and the rats were divided into four groups with four rats in each group. Locally processed short and long species of olive oil was given to two different groups; industrially processed olive oil was also administered according to their body weight. The effects of these oils on their body weight, liver and kidney were accessed through weighing, liver and kidney oxidative stress markers and histological examination. These different administrations were

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compared with a normal control which was not given with any oil. Results of these tests showed a significant difference in the different treatment groups. Therefore, consumption of industrially processed olive oil may constitute a chronic effect on the liver and kidney of its consumers. However, locally processed short species had milder harmful effect compared to locally process long and industrially processed.

Keywords: Olive oil; kidney and liver.

1. INTRODUCTION

Olive (*Olea europaea* L.) oil is a fundamental component of the Mediterranean diet [1]. In the last few decades there has been a significant increase in the global consumption of olive oil, even in countries where it is not produced, such as the Canada and Japan [2]. This is due in large part to its nutritional and health-promoting effects [3], which have been related to the optimal balance between saturated, monounsaturated (MUFA), and polyunsaturated fatty-acids (PUFA), as well as components such as chlorophyll, polyphenols and tocopherols [4]. The importance of olive oil, obtained from a drupe fruit, is increasing due to the biological properties of several of its components that preserve health and prevent many degenerative illnesses [5]. Effectively, olive oil has a beneficial effect on the cardiovascular system, lowering the plasma levels of cholesterol and polyunsaturated fatty acids, and decreasing in the same time the risk for low-density-lipoprotein oxidation, which leads to a healthy lipoprotein profile [6]. Furthermore, olive oil diets help in controlling blood pressure, glucose and lipids levels in diabetic patients [7] as well as in improving the immune function by attenuating the inflammatory process [8].

Previous studies of rats fed with high-fat diets enriched with olive oil showed that the rats achieved low body weights [9] and retained normal insulin sensitivity [10]. In contrast, Buettner et al. [11] found feeding rats a diet high in extra virgin olive oil (EVOO) over a long period of time produces obesity and insulin resistance, similar to what is seen in animals fed a lard-based diet. These findings suggest that obesity and the deleterious effects on vascular integrity of ingesting high quantities of fats may only occur with chronic ingestion. Most of the recent studies on the beneficial effects of EVOO ingestion [12], indicates that despite the numerous benefits it possesses, excess accumulation of these oil will constitute some health complication.

2. MATERIALS AND METHODS

2.1 Assays Used to Examine Oxidative Stress in Disease Conditions

2.1.1 Determination of superoxide dismutase activity

The level of SOD activity was determined by the method by [13].

2.1.1.1 Principle

The ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for this dismutase. Superoxide ($O_2 \bullet^-$) radical generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per $O_2 \bullet^-$ introduced increased with increasing pH and also increased with increasing concentration of epinephrine. These results led to the proposal that autoxidation of epinephrine proceeds by at least two distinct pathways, only one of which is a free radical chain reaction involving superoxide ($O_2 \bullet^-$) radical and hence inhabitable by superoxide dismutase.

2.1.1.2 Procedure

0.2 ml of sample was diluted in 0.8 ml of distilled water to make a 1 in 5 dilution. An aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

2.1.2 Determination of catalase activity

Catalase activity was determined according to the method of Claiborne cited by [14].

2.1.2.1 Principle

This method is based on the loss of absorbance observed at 240 nm as catalase splits hydrogen peroxide. Despite the fact that hydrogen peroxide has no absorbance maximum at this wavelength, its absorbance correlates well enough with concentration to allow its use for a quantitative assay. An extinction coefficient of 0.0041 mM^{-1} cm^{-1} was used.

2.1.2.2 Procedure

Hydrogen peroxide (2.95 ml of 19 mM solution) was pipette into a 1 cm quartz cuvette and 50 µl of sample added (as shown in the table below). This was done to reduce the dilution of the samples (done according to the other protocols whereby H_2O_2 was prepared separately in distilled water (100 mls) and the buffer was also prepared separately.

The mixture was rapidly inverted to mix and placed in a spectrophotometer. Change in absorbance was read at 240 nm every minute for 5 minutes.

2.1.3 Assessment of Lipid Peroxidation (LPO)

Lipid peroxidation was determined by measuring the levels of Malondialdehyde produced during lipid peroxidation according to the method described by [15].

2.1.3.1 Principle

This method is based on the reaction between 2-thiobarbituric acid (TBA) and MDA: An end product of lipid peroxide during peroxidation. On heating in acidic pH, the product is a pink complex which absorbs maximally at 532 nm and which is extractable into organic solvents such as butanol. Malondialdehyde (MDA) is often used to calibrate this test and thus the results are expressed as the amount of the free MDA produced.

2.1.3.2 Procedure

An aliquot of 400 µl of the sample was mixed with 1.6 ml of tris-KCl buffer to which 500 µl of 30% TCA was added. Then 500 µl of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C. This was then cooled in ice and centrifuged at 3000 g for 5 minutes. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. Lipid peroxidation expressed as MDA formed/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ Cm^{-1}$

$$\text{LPO (MDA formed/mg protein)} = \frac{(\text{Absorbance x volume of mixture})}{(E_{532nm} \text{ x volume of sample x mg protein})}$$

MDA FORMED = mmol/mg protein

2.1.4 Estimation of reduced glutathione (GSH) level

The method cited by [16] was followed in estimating the level of reduced glutathione.

2.1.4.1 The principle

The reduced form of glutathione comprises in most instances the bulk of cellular non protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable yellow color when 5', 5'- dithios – (2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman reagent with the reduced GSH, 2-nitro 5-thiobenzoic acid possess a molar absorption at 412 nm. Reduced GSH is proportional to the absorbance at 412 nm.

This method has the following advantages over the earlier modification of the nitroprusside method.

- The precipitation process is carried out with a single easily prepared reagent. It does not require addition of solid sodium chloride or prolonged shaking.
- The determination may be carried out at any temperature likely to be encountered in the laboratory.
- The color formed is relatively stable.
- The sensitivity of the method is so great that it may readily be adapted to a micro-procedure.
- The reagent for colour development is stable for many weeks.

2.1.4.2 Procedure

An aliquot of the homogenate was deproteinated by the addition of an equal volume of 4% sulfosalicyclic acid. This was centrifuged at 4,000 xg for 5 minutes. Therefafter, 0.5 ml of the supernatant was added to 4.5 ml of Ellman reagent. A blank was prepared with 0.5 ml of the

diluted precipitating agent and 4.5 ml of Ellman reagent. Reduced GSH level is proportional to the absorbance at 412 nm.

2.1.5 Determination of protein concentration

The protein concentrations of the various samples were determined by means of the Biuret method as cited by [17] with some modifications: potassium iodide was added to the reagent to prevent precipitation of Cu^{2+} ions as cuprous oxide.

2.1.5.1 Principle

Proteins form a coloured complex with cupric ions in an alkaline solution as exemplified by the Biuret reagent containing $CuSO_4$, KI and sodium potassium tartarate. The protein and Biuret reagent form complex with maximum absorbance at 540 nm. The procedure is usually calibrated with a standard BSA curve.

2.1.5.2 Procedure

The brain supernatants were diluted 10 times with distilled water. This was done to reduce the sensitivity range of Biuret method. 1 ml of the diluted sample was taken and added to 3 ml of Biuret reagent in triplicate. The mixtures were incubated at room temperature for 30 minutes after which the absorbance was read at 540 nm using distilled water as blank. The protein contents of the samples were usually extrapolated from the standard curve and multiplied by the dilution factor to get the actual amount in the fraction.

2.1.6 Aspartate Aminotransferase (AST)

Aspartate Aminotransferase (AST) like ALT is an intracellular enzyme involved in amino acid and carbohydrate metabolism. It is present in high concentrations in the liver and muscle. It is involved in the transfer of amino group from aspartate to α-Ketoglutarate to form oxaloacetate and glutamate.

The diagnostic implications of this enzyme in the serum include liver cirrhosis, myocardial infarction (becoming evident 4-8 hours after the onset of pain and peaking after 24-36 hours), muscular dystrophy and paroxysmal myoglobinuria in which the level is often higher than that of AST. As in ALT, elevated level is also found in metastatic or primary liver neoplasm.

2.1.6.1 Principle cited by [18]

AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

2.1.6.2 Procedure

Briefly, 0.1 ml of sample was mixed with Reagent 1(phosphate buffer, 100 mmol/l, pH 7.4; L-aspartate, 100 mmol/l; and α-ketoglutarate, 2mmol/l) and the mixture was incubated for exactly 30 minutes at 37°C. 0.5 ml of reagent 2 (2,4-dinitrophenylhydrazine (2 mmol/l) was added to the reaction mixture and allowed to stand for exactly 20 minutes at 25°C. Then 5.0 ml of Sodium hydroxide (0.4 mol/l) was added and the absorbance read against the reagent blank after 5 minutes at 546 nm.

2.1.7 Alanine Aminotransferase (ALT)

Alanine Aminotransferase (ALT) is an intracellular enzyme involved in amino acid and carbohydrate metabolism. It is present in high concentrations in the liver and muscle. It is involved in the transfer of amino group from alanine to α-Ketoglutarate to form pyruvate and glutamate. The diagnostic implications of this enzyme in the serum include hepatitis and other liver diseases in which the level is often higher than that of AST. Elevated level is also found in metastatic or primary liver neoplasm.

2.1.7.1 Principle cited by [18]

Alanine Aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

2.1.7.2 Procedure

Briefly, 0.1 ml of diluted sample was mixed with phosphate buffer (100 mmol/L, pH 7.4), L alanine (100 mmol/L), and α-oxoglutarate (2 mmol/L) and the mixture incubated for exactly 30 min at 37°C. 0.5 ml of 2, 4-dinitrphenylhydrazine (2 mmol/L) was added to the reaction mixture and allowed to stand for exactly 20 min at 250 C. Then 5.0 ml of NaOH (0.4 mol/L) was added and the absorbance read against the reagent blank after 5 min at 546 nm.

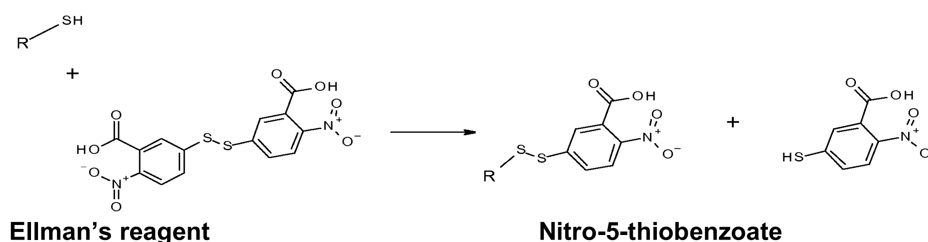

Fig. 1. Mechanism of action of reduced GSH with Ellman's reagent (Beutler et al., 1963)

2.1.8 Estimation of serum albumin

The determination of serum albumin cited by [19] was carried out using colorimetric method.

2.1.8.1 Principle

Albumin reacts with the dye-bromocresol green which has specific affinity for it in acidic solution to produce a coloured complex which is measured colorimetrically. The intensity of this colour is proportional to the concentration of albumin in the sample.

2.1.8.2 Procedure

Two test tubes labeled test and standard had 4.0 ml of working coloured solution dispensed into them. To the test tube labeled test, 0.02 ml of serum was added and 0.02 ml of standard to the standard test tube. The test tube contents were separately mixed and incubated at 37°C for 10 minutes. The spectrophotometer was set to zero using working BCG. The absorbance of test and standard were read at 628 nm.

2.1.9 Histology

The effect of olive oil on histological structures of liver and kidney of albino rats were analysed to determine the integrity of the cells after administration.

2.1.9.1 Procedure

After sacrifice of animals, part of the liver and kidany tissues from each animal from treated and control was removed and immersed in 10% buffered formalin solution. Each part of liver and kidney tissues were kept in separate numbered small glass bottles and then embedded in paraffin, and sectioned. Four sections (5 microns in thickness) were taken from each liver and kidney tissues, each section being at a distance of at least 500 u from the proceeding one sections were stained with haematoxylin and eosin [20].

2.2 Statistical Analysis

The data are presented as mean ± S.D. Statistical analyses between control and treated animals were performed using graph pad prism statistical tool.

3. RESULTS

3.1 Weight and Analysis of Variance

Below are the results of weight gain and weight loss after administration of the different species of olive oil and control.

Table 1. Weight difference of rats before and after administration

Treatment	Initial Weight (g)	Final Weight (g)	Weight Difference (g)
C_1	200	210	+10
C_2	205	219	+14
C_3	201	215	+16
C_4	192	211	+19
IPP_1	200	193	-7
IPP_2	200	194	-6
IPP_3	180	173	-7
IPP_4	205	198	-7
LSP_1	205	199	-6
LSP_2	220	214	-6
LSP_3	210	202	-8
LSP_4	180	169	-11

Treatment	Initial Weight (g)	Final Weight (g)	Weight Difference (g)
LLP_1	205	197	-8
LLP_2	185	180	-5
LLP_3	190	184	-6
LLP_4	200	195	-5

Key: C= Control, IP= Industrial Processed, LS= Local Short Processed, LL= Local Long Processed

Table 2. Kidney function test of rats after administration

Treatment	GSH	MDA	CAT	SOD	PROT
C	19.73±0.41	3.27±0.05	595.87±6.02	100.12±6.28	27.16±1.47
IP	14.12±1.16[a]	6.46±0.60[a]	2270.53±59.15[a]	611.53±45.93[a]	26.53±2.16[a]
LL	11.43±1.28[ab]	5.21±2.23[ab]	1028.46±490.89[ab]	325.00±183.26[ab]	34.62±9.11[ab]
LS	13.31±1.59[ab]	4.77±1.65[ab]	1200.64±247.27[ab]	301.92±177.46[ab]	34.93±7.36[ab]

Values are expressed as Mean ± SD, n= 4 for each group.
[a]Values are significantly different when compared with control (p<0.05).
[b]Values are significantly different when compared with control (p<0.05)

Table 3. Liver function test of rats after administration

Treatment	GSH	MDA	CAT	SOD	PROT
C	71.73±0.81	2.65±0.03	903.67±1.00	357.69±3.14	79.09±51.46
IP	31.37±6.21[a]	6.66±1.41 [a]	884.44±15.83 [a]	296.73±35.07 [a]	28.86±1.02 [a]
LL	51.06±15.09[ab]	2.97±1.73[ab]	675.83±212.25[ab]	240.38±52.68[ab]	35.95±3.54[ab]
LS	56.31±19.00[ab]	3.00±2.58[ab]	705.99±120.78[ab]	269.23±43.05[ab]	32.65±2.05[ab]

Values are expressed as Mean ± SD, n= 4 for each group.
[a]Values are significantly different when compared with control (p<0.05).
[b]Values are significantly different when compared with control (p<0.05)

Table 4. Serum test of rats after administration

Treatment	AST (U/L)	ALT (U/L)	TP (g/L)	ALB (g/L)
C	101.47±1.29	19.89±0.55	72.64±5.42	30.44±4.51
IP	102.73±37.63[a]	14.17±6.98 [a]	73.33±3.68 [a]	35.92±1.81 [a]
LL	96.19±6.22 [ab]	13.61±5.08 [ab]	69.93±2.46 [ab]	36.83±4.09 [ab]
LS	105.88±8.07 [ab]	16.26±3.00 [ab]	74.32±3.98 [ab]	38.31±8.95 [ab]

Values are expressed as Mean ± SD, n= 4 for each group.
[a]Values are significantly different when compared with control (p<0.05).
[b]Values are significantly different when compared with control (p<0.05)

4. DISCUSSION

In this study, hepatic, kidney antioxidant enzymes (SOD, CAT, and GST) activities significantly increased in rats of treated groups compared to those in control group. The increase in the amount of these enzymes could be attributed to non-utilization of these enzymes in scavenging the free radicals generated due to the high cholesterol in the oils or insufficient antioxidant enzymes in the kidney.

Superoxide dismutase, Catalase, Glutathione-s-transferase, malonaldehyde and Gluthione peroxidase constitute a mutually supportive team of defense against Reactive Oxygen Species (ROS). Glutathione is a small tripeptide protein synthesized in the liver [21]. It is a potent antioxidant with high redox potential and it also serves as a co-factor for several oxidative stress detoxifying enzymes (glutathione peroxidase and glutathione transferase) [22,23]. Depletion of GSH has been reported in apoptosis and many degenerative conditions [23]. The results of the present study showed that the level of glutathione (GSH) was significantly decreased ($p < 0.05$) in the liver of the rats fed with industrially process olive oil, long process and short process species compared to the control group. The reduction is more eminent in the industrially process olive oil when compared with control than in long and short locally process. This observation is consistent with administration of high cholesterol in experimental rats [24]. It can be assumed that the reduction in tissue glutathione levels was as a result of increased oxidative stress and lipid peroxidation occasioned by the high cholesterol diet [25,26].

A lot of studies have reported that increased aldehydes such as malondialdehyde (MDA) and conjugated dienes are involved in hyperlipidemia-provoked free radical attacks on membrane lipoproteins and polyunsaturated fatty acids [27]. In this study, the increase in MDA in industrially process olive oil and short species indicates that there was an injury in liver caused by high cholesterol in these oils.

In this study, hepatic antioxidant enzymes (SOD and CAT) activities significantly decreased in the treated groups compared to the control group. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady state level of O_2^-.Under normal conditions, antioxidant enzymes such as superoxide dismutase (SOD) catalyse the conversion of superoxide radicals (O_2-) into hydrogen peroxide (H_2O_2) and O_2 [28] and catalase further detoxifies H_2O_2 into H_2O and O_2 [29] while glutathione peroxidase also functions in detoxifying H_2O_2 like catalase and GST plays an essential role in the liver by eliminating toxic compounds by conjugating them with GSH. However, imbalance between the formation of reactive oxygen species and their elimination occasioned by hypercholesterolemia has been implicated in oxidative-induced diseases. The decrease in the activities of (SOD) could be attributed to the excessive utilization of these enzymes in inactivating the free radicals generated due to the high cholesterol in the oils [30] or insufficient availability of GSH. This observation is further substantiated by the elevated malondialdehyde levels.

Serum enzymes including AST, ALT and ALP are used in the evaluation of hepatic disorders. An increase in these enzyme activities reflects active liver damage. In this study, an increase in AST in the industrially process olive oil and short species when compared with control group showed that there was significant liver damage in the rats. The short species gave a slight reduction in the activity of the enzyme. ALT enzyme activity was normal in all the samples administered. Evaluation of total protein or albumin status may be helpful in the assessment of disease progression [31]. Albumin is an important component of plasma antioxidant activity that primarily binds free fatty acids, divalent cat ions and hydrogen oxychloride (HOCl) [32]. Albumin is the most abundant protein in human plasma, representing 55-65% total protein. It synthesized in the liver at a rate that is dependent on protein intake subject to feedback regulation by the plasma albumin level. Little albumin is filtered through the kidney glomeruli and most of that is reabsorbed by proximate tubule cells and degraded by their lysosomal enzyme into fragments that are returned to the circulation. In this study, there was a significant decrease in serum albumin of rats in all the groups compared to control, indicating poor liver function.

The aminotransaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] represent an important link between carbohydrate and amino acid metabolic pathways [33]. Also, these enzymes are considered good sensitive tools for detection of any variations in the physiological process of living organisms [34,35]. The concentrations of these enzymes (AST & ALT) in liver of treated rats were significantly increased than that in control rat. The elevation in the activities of these enzymes could be due to a variety of conditions including muscle damage, intestinal and hepatic injury and toxic hepatitis [36]. As well, the increase in serum alkaline phosphatase (ALP) activity in patients could be attributed to hepatocytes injury and interruptions in their natural activities [36].

In Fig. 1, which is a tissue of normal rat which was not administered olive oil, right arrow shows a normal glumerulei, down arrow shows normal nuclei within the collecting duct, signifying that no damage was done to the this tissue. Fig. 2: Kidney of rat administered industrially processed olive oil; right arrow shows mild eroding of the epithelial lining of the glumerulei and down arrow shows partial dislodgement of nuclei within the collecting duct Fig. 3: Kidney of rat administered long local species processed olive oil, down arrow shows distortion of the epithelial lining of the glomerulei and up arrow shows distortion of the nuclei within the collecting duct. Fig. 4: Kidney of rat administered short local species processed olive oil, right arrow shows a normal epithelial lining within the glumerulei and left arrow shows a normal nuclei within a collecting duct. Fig. 5: Liver of rat administered water (control), down arrow shows normal nuclei within the hepatocyte and up arrow shows a normal radial arrangement of hepatocyte with presence of kuppfer cell within the sinusoid. Fig. 6: Liver of rat administered industrially processed olive oil, up arrow show a mild enlarge nuclei within the hepatocyte and down arrow shows mild distortion of the radial arrangement of hepatocyte with presence of kuppfer cell within the sinusoid.

Fig. 7: Liver of rat administered long local species processed olive oil, down arrow shows massive enlargement of nuclei within the hepatocyte and up arrow shows mild distortion of the radial arrangement of hepatocyte with presence of few kuppfer cells. Fig. 8: Liver of rat administered short local species processed olive oil; right arrow shows a normal radial arrangement of hepertocytes and down arrow shows normal nuclei within the hepertocyte.

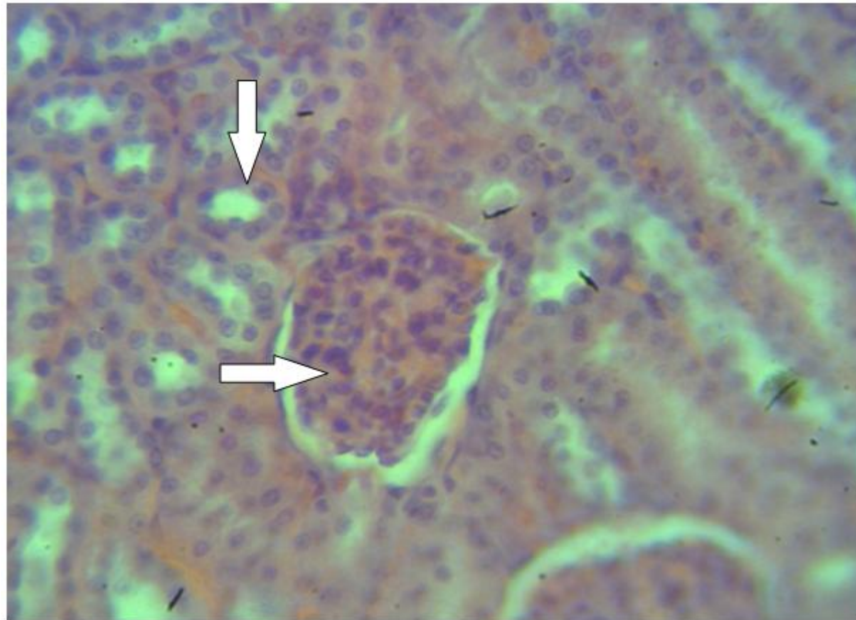

Fig. 1. Kidney of rat administered water (control)

- *Right arrow shows a normal glumerulei*
- *Down arrow shows a normal nuclei within the collecting duct*

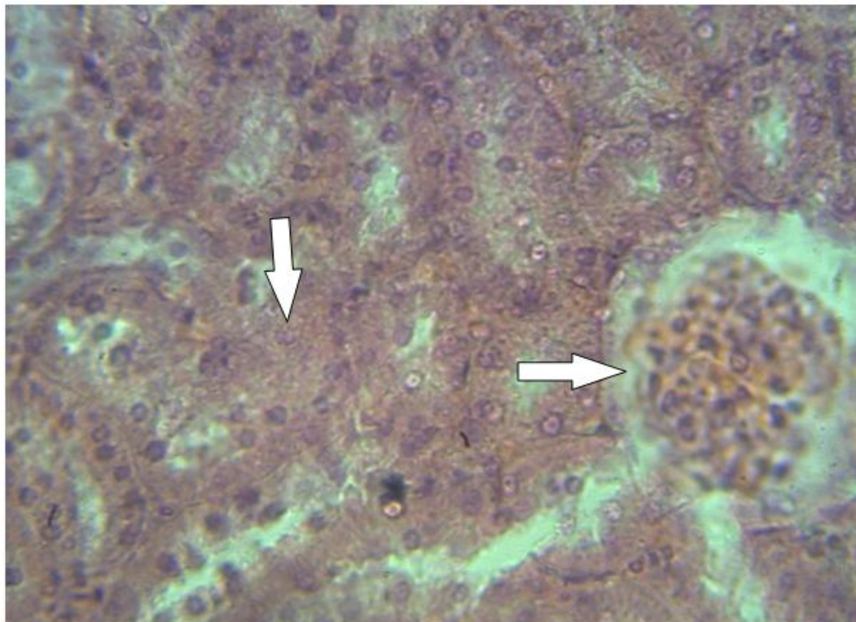

Fig. 2. Kidney of rat administered industrially processed olive oil

- *Right arrow shows mild eroding of the epithelial lining of the glumerulei*
- *Down arrow shows partial dislodgement of nuclei within the collecting duct*

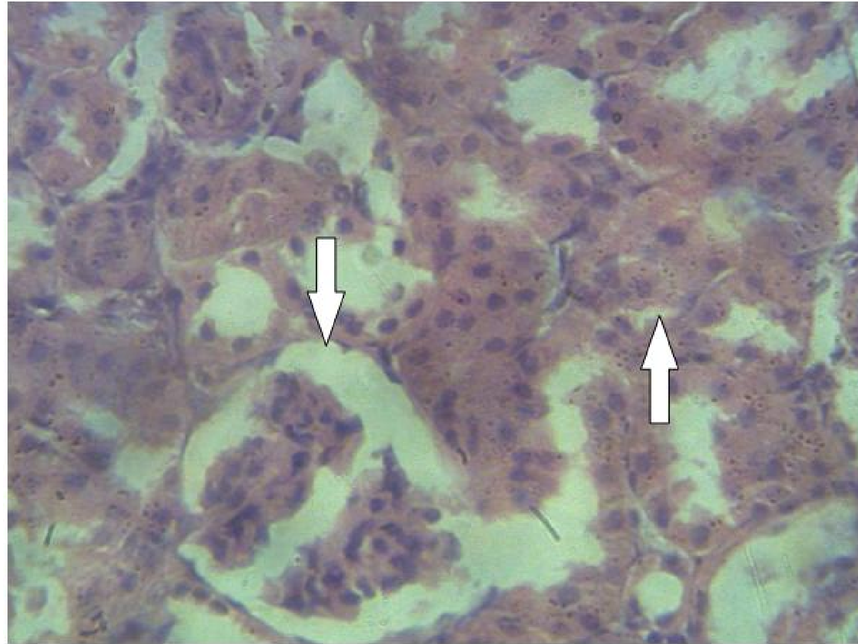

Fig. 3. Kidney of rat administered long local species processed olive oil

- *Down arrow shows distortion of the epithelial lining of the glomerulei*
- *Up arrow shows distortion of the nuclei within the collecting duct*

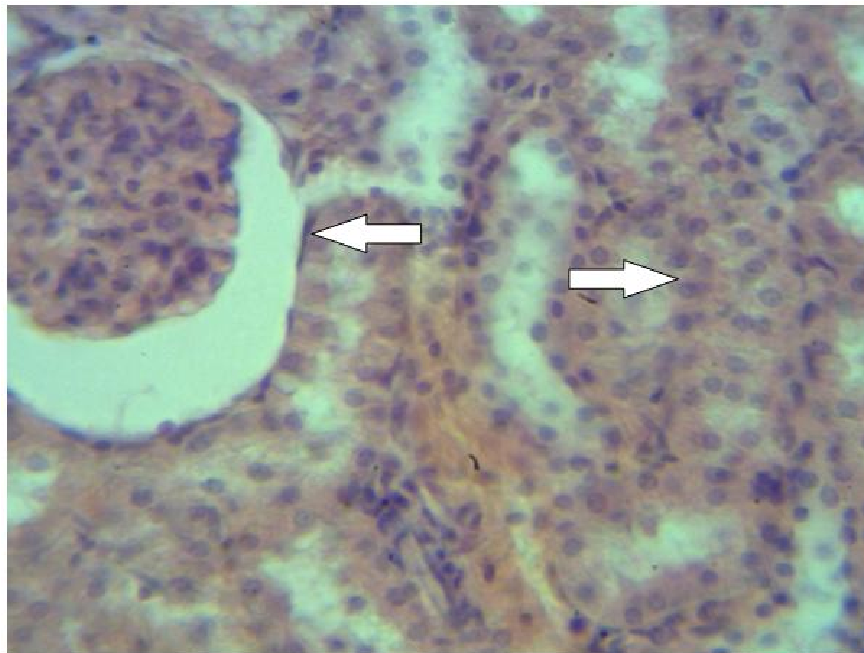

Fig. 4. Kidney of rat administered short local species processed olive oil

- *Right arrow shows a normal epithelial lining within the glumerulei*
- *Left arrow shows a normal nuclei within a collecting duct*

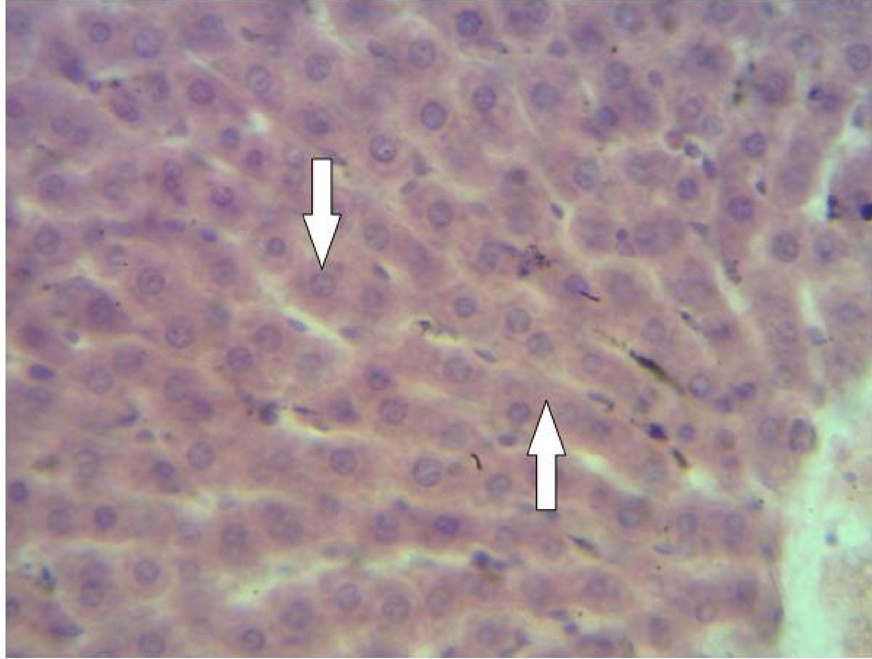

Fig. 5. Liver of rat administered water (control)

- *Down arrow shows a normal nuclei within the hepatocyte*
- *Up arrow shows a normal radial arrangement of hepatocyte with presence of kuppfer cell within the sinusoid.*

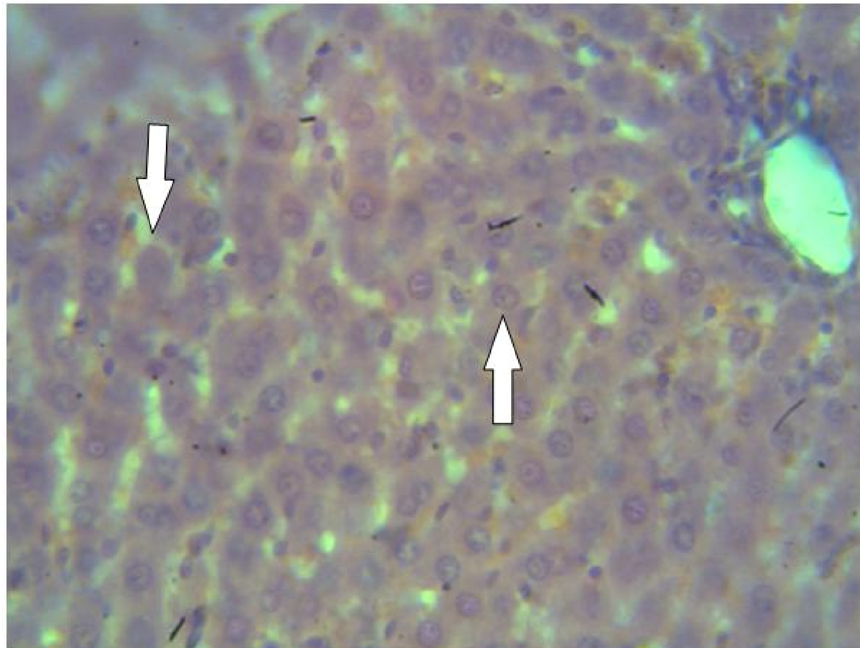

Fig. 6. Liver of rat administered industrially processed olive oil

- *Up arrow show a mild enlarge nuclei within the hepatocyte*
- *Down arrow shows mild distortion of the radial arrangement of hepatocyte with presence of kuppfer cell within the sinosoid*

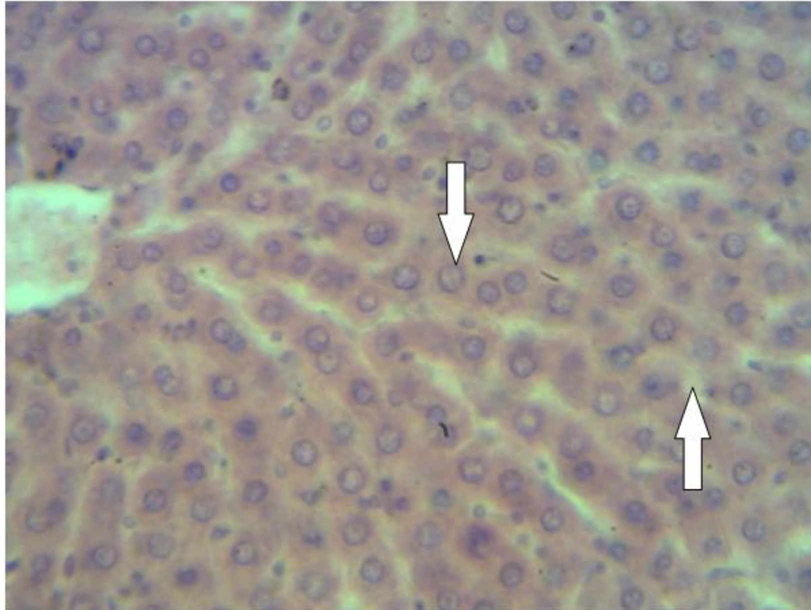

Fig. 7. Liver of rat administered long local species processed olive oil

- *Down arrow shows massive enlargement of nuclei within the hepatocyte*
- *Up arrow shows mild distortion of the radial arrangement of hepatocyte with presence of few kuppfer cell*

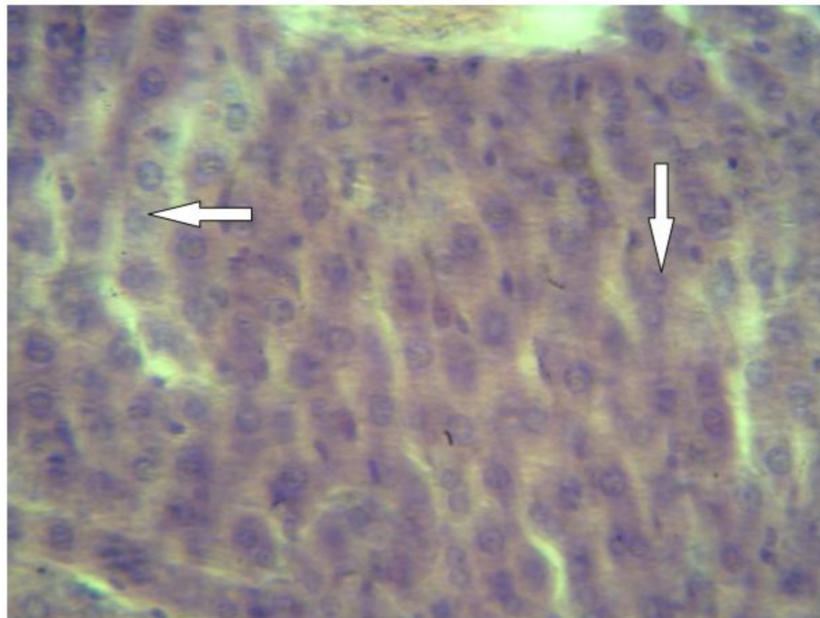

Fig. 8. Liver of rat administered short local species processed olive oil

- *Right arrow shows a normal radial arrangement of hepertocytes*
- *Down arrow shows a normal nuclei within the hepertocyte*

The pictures and discussion of the histological results of kidney and liver tissues above showed that consuming industrially processed and long locally processed olive oil have destructive effect on tissues while short locally processed olive oil has no destructive effect on tissues.

5. CONCLUSION

Despite the healthy nature of olive oil, its prolong ingestion can constitute a chronic side effects to its consumers. Therefore, moderation should be observed when consuming olive oil, so as to avoid any chronic side effect. In addition, consumption of locally processed olive oil as it is practiced in rural villages is encouraged due to its little damaging effects on tissues of liver and kidney compare to industrially process one. Furthermore, the long locally processed species has showed a significant harmful effect on tissues compared to the industrially processed and short locally processed species. Thus, industrially processed and long locally processed indicated more tissue damaging effect. Therefore, excessive consumption should be discouraged so as to ensure a healthy population in the future. Consequently, more research on this area are encouraged, to determine the extent of damage done to tissues by these olive oils.

ETHICAL APPROVAL

As per international standard or university standard ethical approval or this study has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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