IMPACT OF EDIBLE OIL ON SERUM CYTOKINE AND EXPRESSION PATTERNS OF GENES HMGCR AND G6PDH IN RABBIT LIVER.

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Abstract:
This research work was conducted to study the effect of edible oil on serum pro inflammatory cytokines (IL-6 and TNF-α) and anti-inflammatory cytokine (IL-10), Liver histological changes in experimental rabbits and HMGCR and G6PDH gene expression pattern in rabbit liver. Edible oils are generally used in home cooking and industrial food manufacturing worldwide and are the major source of unsaturated fats in human diets. Moreover, as with all food products, there is a probable for potentially toxic contaminants to occur in oils. An experimental study design was conducted eighteen albino rabbits were fed with rabbit food pallets for 21 days, of which 06 were fed normal diet (Group-I), and the other 06 were fed with edible oil at 2 ml/kg + normal feed (Group-II), third group of 06 rabbits were fed edible oil at 5 ml/kg + normal feed (Group-III). Estimation of cytokines levels in serum and the expression levels of HMGCR and G6PDH genes in rabbit liver were determined by using real-time fluorescent quantitative PCR and Western blot. The expressions of HMGCR and G6PDH mRNAs, protein levels and activity in Group-II and group - III were significantly over expressed as compared with those in Group-I. Low levels of SOD and CAT were observed in liver of Group -III rabbits compared with Group-II and Group-I. It was concluded that edible oil showed regulative effect on liver by a potential mechanism of high expression HMGCR and G6PDH genes, protein abundance, activity of HMGCR and G6PDH and low concentrations of SOD and CAT in serum, consequently histopathological alterations are observed in edible oil experimental rabbit liver.

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1. Introduction:

Palm oil is an edible oil acquire from the mesocarp (reddish pulp) of the fruit of the oil palms, rich in b-carotene and commonly consumed in Southern Nigeria **(1)** and the oil is readily digestible and absorbed **(2).** In order to reveal more about the effect of edible oil on cholesterol metabolism in the body, has conducted research on the effects of various concentrations of oil against oxidative stress and inflammation in male rabbits. The high content of saturated fatty acids (SFA) and particularly of palmitic acid has been the focus of attention concerning their effects on cardiovascular diseases and coronary heart disease **[3, 4].** Edible oil used as research material, because the oil contains a lot of saturated fatty acids, unsaturated and unsaturated fatty acids which have been transformed into trans fatty acids that are dangerous to health. Though these effects of diets have been abundantly documented, fewer studies have been devoted to the effect of palm oil on liver lipid metabolism. Yet, sedentary lifestyle associated with excess fat consumption contributes in a growing number of countries to the development of obesity-related disorders, among which fatty liver disease is an important part **[5].** Indeed, non-alcoholic hepatic steatosis, that may generally be accompanied with oxidative stress and inflammation, occurs in the liver, when the rate of uptake and synthesis of fatty acids exceeds the rate of oxidation and secretion, which is generally the case with high-fat diets (HFD) **[6].** In addition, dietary fats with different lipid composition may differently influence the liver response and lipid accumulation **[7].** In the Mediterranean region, a lower prevalence of cardiovascular diseases has been observed and partially attributed to a regular intake of edible oil **[8,9].** This has been the basis for a significant number of studies concerning health benefits of edible oil **[10-12].** but regardless of all these studies, the practice seems to continue. Palm oil is equally composed of saturated and unsaturated fatty acids. It principally contains saturated palmitic acid which changes serum cholesterol reported by **Keys et al. (1965) (12).**

Oxidative stress is a condition in which the amount of free radicals in the body exceeds the capacity of antioxidants in the body so that the body cannot neutralize them. As a result, the intensity of the oxidation process of normal body cells becomes higher and cause more severe damage **(13).** Levels of free radicals exceeds of the antioxidant produced will cause imbalance resulting in oxidative stress. Dyslipidemia may increase oxidative stress **(14-16)** and reduced antioxidant defenses **(17-19)**. Increased oxidative stress contributes to vascular disorders, inflammatory function, atherosclerosis thrombosis, and eventually cause disease of blood vascular **(20).** Malondialdehyde (MDA) unstable compounds from the decomposition of lipid peroxide as a result of reactions between free radicals with unsaturated fatty acids and is one of the parameters of oxidative stress in the body.

A number of other biochemical mediators also act to initiate and perpetuate the inflammatory reaction. Reactive oxygen species (ROS) may perpetuate inflammation by facilitating the generation of chemotactic factors at the local site **[21-23].** Superoxide anion radical, the peroxynitrite anion and the hydroxyl radical are the major ROS generated during the disease condition **[24, 25].**

Fatty acid especially as ester the natural oils and fats but there can be in the form of free fatty acids esterified as that is a form transfer contained in plasma. Fatty acids contained in natural fats usually are straight-chain derivatives containing an even number of carbon atoms. The chain can be saturated (containing no double bonds) or unsaturated (containing one or more double bonds). Effect of dietary saturated fatty acids and cholesterol may increase serum cholesterol can lead to atherosclerosis which is a major factor in the occurrence of premature cardiovascular disease **(26, 27).**

HMGCR is one of the crucial enzymes in cholesterol biosynthesis **(28).** HMGCR transcription is coordinated by Sterol-regulatory element binding proteins (SREBPs), of which SREBP2 is the leading subtype that regulates liver cholesterol **(29, 30).** Meanwhile, the content of cholesterol in the cell can affect the activity of HMGCR forming a feedback loop that forms the homeostasis of cholesterol **(31).** Epigenetic regulation including DNA methylation and histone modification on foetal gene expression during cholesterol metabolism have been well demonstrated **(32).** 3-hydroxy-3-methyl-glutaryl-coenzyme - A reductase (HMGCR) was a microsomal enzyme that control the rates of intracellular cholesterol esterification and synthesis, respectively. These two enzymes, which regulate the amount of unesterified cholesterol within a cell, are responsive to changes in microsomal lipid composition. This work was explained to estimate the effects of edible oil on liver biomarker genes linked to cytokine

regulation, antioxidative stress and more generally to cytokines in blood serum. The novelty of this work is the comparison in different concentrations of the metabolic effects of edible oil the later being considered as an important ingredient of the healthy Mediterranean diet. We hypothesized that edible oil treated rabbits would induce an increase in serum IL-6 and IL-10, and serum TNF-α as well as a consequent increase in antioxidants.

2. Materials and methods:

2.1 Experimental Design:

Eighteen albino rabbits were fed with edible oil and rabbit food pallets for 21 days, of which 06 were fed normal diet (Group-I), and the other 06 were fed with edible oil at 2 ml/kg + normal feed (Group-II), third group of 06 rabbits were fed edible oil at 5 ml/kg + normal feed (Group-III). Over experimental period, groups of animals were sacrificed and blood samples were obtained to quantify TNF-α, IL-10, IL-6 and Liver samples used for antioxidative enzyme analysis SOD, CAT and gene expression analysis of HMGCR, G6PDH for each group. Statistical analyses included mean, standard deviation, ANOVA tests.

2.2. Superoxide dismutase (SOD- EC: 1.15.1.6,)

Superoxide dismutase activity was determined by simple and rapid method, based on the ability of the enzyme to inhibit the auto-oxidation of pyrogallol according to the method of Marklund *et al.* (1974)**.** 50µl of enzyme source (10% homogenate) was added to 1ml of (0.1 M) Tris HCL buffer. To this, 1ml of 0.2M pyrogallol solution and 50 µl of distilled water were added. Absorbance was read at the wavelength of 420 nm against Tris-EDTA buffer at zero time and after 1 minute of the addition of pyrogallol. SOD activities were expressed as mmoles epinephrine oxidized/mg protein/min.

2.3. Catalase (CAT- EC: 1.11.1.6)

Catalase activity was measured by slightly modified method of Aebi (1984) **(33)** at room temperature. 100µl of enzyme source (10 % homogenate) was added with 10µl of ethanol and then placed in an ice water-bath for 30 min and were kept at room temperature followed by the addition of Triton-X 100. The reaction mixture was prepared by adding 200µl of phosphate buffer (50 mM, pH 7.0), 250 µL of 0.066 M hydrogen peroxide and 50µL enzyme source. The decrease in absorbance was measured at wavelength of 240 nm for every 10s intervals for 3 min against blank containing all the components except the hydrogen peroxide. The activity of enzyme was expressed in µmol of hydrogen peroxide metabolized/mg protein/min. The molecular extinction coefficient of 43.6M/cm was used to determine the catalase activity. One unit activity is equal to the µmoles of H_2O_2 degraded/ mg protein/ min.

2.4 Serum levels of Pro inflammatory cytokines (IL-6 and TNF-α) and anti-inflammatory cytokine (IL-10) :

A total of 5 ml blood was collected from experimental rabbits in red vacutainer and centrifuged at 600 rpm for 10 min. The serum was separated and stored at -80°C in cryovials until analysis. To analysis the levels of IL-10, TNF-α, IL-6 presented in serum, an enzyme-linked immunosorbent assay (ELISA) was acomplished using commercially available kits for cytokine detection (for rabbit TNF-α, IL-10, IL-6). The preparation of all reagents, the working standards, and protocol were followed according to the manufacturer's instructions. The absorbance was read using ELISA reader (BIO-RAD) at 450 nm and 570 nm dual filters. All the samples were thawed only once and assayed in duplicate.

2.5 Histological changes

After cervical dislocation of rats, Liver was collected from all 3 groups. The tissues were fixed in neutralized formalin, dehydrated with ethanol and embedded in paraffin wax (56 °C). Some tissues were made into thin sections and stained with haematoxylin and eosin. The stained sections were viewed under microscope and the histological changes were recorded with the help of a pathologist.

2.6 RNA extraction and cDNA synthesis:

Liver samples were snap-frozen in liquid nitrogen. Each sample was synthesized from total RNA was extracted from liver tissue using Trizol Reagent (Thermo fisher Scientifics) in accordance with the protocol provided by applied bio systems. RNA concentrations were quantified by measuring the optical density at 260-nm wavelengths using the Nanodrop ND-1000 spectrophotometer. Purity was determined as the 260/ 280 nm ratio with expected values between 1.8 and 2.0, indicating absence of protein contamination. The reverse transcription step was conducted on 500 ng of RNA using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit.

2.7 qRT PCR Analysis:

To measure expression of HMGCR and G6PD mRNA of liver, real-time PCR was performed using a Real Time PCR System (BIO-RAD

CFX384 Touch Real-Time PCR System). The mRNA expression of HMGCR forward primer (5'-CCGTTGACTGAAAATGAGCGG -3') and the reverse primer (5'- GGACACACA AG CAGGGA AGA -3') were designed according to HMGCR sequence (Genebank accession No. XM-051838684). The forward primer (5'-AACATA TGAGCAGGGTCCCG-3') and the reverse primer (5'-CTTCTCGTAGGATCCCGCAC-3') were designed according to G6PD sequence (Genebank accession No. XM-008250287). β -actin was used as the internal control gene. The forward primer (5'-ATCG TG GGG CGCCCCAGGCATCAGG-3') and reverse primer (5'-CCTGATGCCT GGG GCCCACGAT-3'). The Amplification of cDNA samples was carried out using the SYBR green PCR kit (Bio-Rad, USA). Fluorescent emission data were captured and mRNA levels were analyzed using the critical threshold (CT) value. Thermal cycling and fluorescence detection were conducted using the Bio-Rad IQ50 sequence detection system (Bio-Rad, USA).

2.8 Tissue Protein Extraction and Western Blot Analysis

Extract total and nuclear proteins from 100 mg of frozen liver tissues were homogenised, as previously described (30). Detection of protein concentration with Pierce BCA Protein Assay Kit (Thermo Scientific, USA). Western blot analysis for HMGCR and G6PDH were follow the manufacturer's instructions. HMGCR and G6PDH was normalized with β-actin.

2.9 Western Blot Analysis

Western blot analysis was performed as previously described (34). The liver tissues were homo-genized in phosphate-buffered saline (PBS) with protease inhibitor cocktail (Calbiochem, San Diego, CA, USA) and the protein concentration was quantified by the Bradford protein assay. The samples (30 µg protein/lane) were mixed with sample buffer, boiled for 6 min, separated by SDS-polyacrylamide (8-15%) gel electrophoresis and then electroblotted onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were then blocked with 5% non-fat dry milk in Tris-buffered saline with Tween-20 buffer (25 mmol/l Tris, pH 7.5, 150 mmol/l NaCl, and 0.1% Tween-20) for 1 h and then incubated overnight at 4˚C with rabbit anti-phospho HMGCR and rabbit anti-phospho-G6PDH antibodies (Cell Signaling Technology, Danvers, MA, USA). The blots were washed with Tris-buffered saline with Tween-20 buffer and incubated with horseradish peroxidase-conjugated anti-rabbit IgG. Signals were visualized using a chemiluminescent detection kit according to the manufacturer's instructions (Amersham Pharmacia Biotech, London, UK). The membranes were then re-probed with rabbit HMGCR and G6PDH antibodies (Cell Signaling Technology) to verify equal loadings of protein in each lane. Analysis of quantitative image was accomplished using NIH Image software (Image J) to determine the concentration of the protein signal, which was expressed relative to the amount of β actin used as an internal control.

2.10 Statistical analysis:

All data were analysed by one-way analysis of variance and Turkey's multiple test to compare treatment means. Differences were considered significant at ≤ 0.05 probability level for all data. All analysis was performed using the GraphPad Prism V.5.03 and results presented as mean ± standard error of the mean (SEM).

3. Results:

3.1 TNF-α, IL-6 and IL-10 levels in blood serum:

Therefore, we determined the circulating levels of TNF-α, IL-6 and IL-10 in response Data in **Fig1** showed that the differences in IL-6, IL-10 and TNF-α concentrations between treatment groups and control groups, but IL-6 level was significantly($P<0.001$) higher by Group-II than Group-III. However, TNF-α concentration was significantly ($P<0.001$) decreased in group-III rabbits compared to control and Group-II rabbits. Concerning Il-10, dietary oil supplementation treated with 5mg (Group-III) significantly increased concentrations with Group-II and Group-III compared to control.

3.2 SOD and CAT activity in Liver:

In (Fig 2) studies in animal the activity of SOD in control rabbits was found to be (10.23 mg protein / min) in liver. In the Group-II, where rabbits were control but treated with 2ml/Kg oil the activity was showed decreased in liver when compared to control (6.89 mg protein/min). In Group-III, Edible oil treated with 5ml/Kg the activity was found significantly decrease (4.93 mg protein/min) over control and Group-II Rabbits.

Catalase (CAT) activity levels in the liver tissues were found to be significantly decreased in the edible oil treated groups. In control rats was found to be (60.28 mg protein/min). In Group-II, the activity was slightly decreased with significant change. Group-III rabbits also Catalase the activity

showed decreased levels when compared to control rats. Finally, Group-II and Group-III rabbits shows high antioxidant disturbances showed in Fig 2 when compared to Group-I.

Histological analysis:

Histological examination revealed that liver cells from experimental animals fed diets Group-I was normal. Liver tissue from group-II rabbits showed alteration of hepatocytes in most livers. Liver cells from animals of group III were abnormal and showed many signs of toxicity, including disintegrated cell walls, and abnormal, folded bile-duct walls. Typical liver cells from animals on each of the two concentrations of edible oil diets are shown in Fig 3.

3.3 Gene Expression Profiles:

Fig 4 showed the hepatic mRNA levels of rabbits after 21 days of experimental period. HMG-CoA reductase and G6PDH were rate-controlling enzymes of cholesterol biosynthesis. The mRNA level of HMG-CoA reductase and G6PDH were increased, however without statistical significance in the edible oil fed rats in comparison with the control-diet rats. mRNA levels of the two enzymes were significantly increased in the experimental groups (Group-II and Group-III) as compared with the controls. The result conferred here showed the improvement of cholesterol hydroxylation, esterification and the conquering of hepatic cholesterol synthesis. The mRNA levels were significantly higher in the rabbits treated with edible oil compared with those control rabbits, notably in Group-III rabbits. Control rats showed lower HMG-CoA reductase and G6PDH mRNA expression than the Group-II and Group-III rabbits.

3.4 Western blot analysis:

To reveal the effects of different concentrations of edible oil on HMGCR and G6PDH protein expression level shown in **fig 6**. increased protein expression level was observed in group-III and group-II compare to control group. But compare to group-II and group -III treated with 5ml edible oil rabbits showed highest upregulation of protein expression. Moreover, we observed higher expression of HMGCR and G6PDH protein level in group-III compared to other two experimental groups.

4. Discussion:

The statement from the present study supports the assumption that IL-6 is an anti-inflammatory cytokine. This is suggested by its very high plasma concentrations observed during infections and by its release from contracting skeletal muscle during Edible oil treated period. In this study, we show that an acute experimental elevation of edible oil a transient increase in the plasma levels of the cytokines IL-6 and decreased levels of IL-10 moreover, edible oil caused a delayed increase in TNF – α. IL-6 serve as a marker for inflammation in visceral fat and in atherosclerotic lesions **(35, 36).** Phenolic compounds owned edible oil is very meaningful change production of cytokines or antioxidants, resulting in barriers to the production of IL-6 and IL8 (Gaulliard, et al., 2008). We observed in this experiment increased concentrations of IL-6 and TNF – α in group-II and Group-III rabbits. Increased level of oxidative stress on a diet high in long chain saturated fatty acids that have the highest composition of palmitic acid appears in the delivery of edible oil. Moreover, plasma levels of IL-6 have been shown to predict total and cardiovascular mortality in population-based studies **(37).** However, chronical elevation of IL-6 is causally associated to obesity, atherosclerosis, and insulin resistance. Based on previous studies stated that IL-6 exerts anti-inflammatory effects **(38),** it is likely that the elevated plasma IL-6 found in group-II rabbits represents low-grade inflammation, rather than group-III rabbits. In this context IL-6 plays a central role in the generation of regulatory T cells imbalance associated with obesity and hypertension. Furthermore, in hepatic inflame-mation both TNF- α and IL-6 are related to rabbits affected with edible oil. Our results showed edible oil induced inflammatory responses including serum TNF- α and IL-6 levels which might be associated with down regulating anti-inflammatory molecules IL-10. As noted, higher IL-6 levels are predictive of increased cardiovascular risk in the elderly; this may reflect a mediating role for IL-6 in cardiovascular disease**(35).**

In contrast to the control group, SOD, CAT levels are decreased in experimental animals. Antioxidant balance is most important for normal biological functioning of the cells and tissues. The present study observed SOD is an enzymatic antioxidant which catalyzes the transformation of super oxide radical to hydrogen peroxide (not a free radical itself, but a reactive molecule) and molecular oxygen. Decreased activities of enzymatic antioxidants such as SOD have been well stated in edible oil treated rats. In normal conditions SOD activity would be parallel to the amount of super oxide radicals produced. In case of Group-II and Group-III the significant decrease in its activity was due to various concentrations of the edible oil treated rabbits. It induces liver

toxicity through the generation of free radicals in high concentration.

Similarly in liver toxicity rabbits the activity of CAT was reduced and this showed a number of deleterious effects due to accumulation of hydrogen peroxide. CAT, which is present virtually in all mammalian cells of mitochondria, is responsible for the removal of H_2O_2 **(39).** The decreased activities of SOD and CAT may be due to the concomitant increase in the generation of free radicals.

The histological assessment of liver sections supports the results obtained from antioxidative analysis in group-II and group-III rabbit livers. The changes observed in hepatic cells by free radicals resulting from edible oil toxicity as a result of cellular injury exist by alteration in membrane permeability. Liver tissues section of hypercholesterolemic rabbits revealed the liver participates in uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides **(41).** The notable changes seen were: dilation and congestion of the hepatic vascularity, inflam-mation, necrosis was statistically evaluated in group-III rabbits.

The reactions elicited is to exclude or limit the increase of injurious agents as well as to remove the consequent cells and tissue. Oxygen derived metabolites are discharged from activated superoxide, hydrogen peroxide, and hydroxide radicals. These leads to endothelial cell damage, increased vascular permeability, metabolic disturbances and finally hepatocyte necrosis. The latter is either a direct cause or a means of reversible injury. Previous studies were demonstrated excessive fat and cholesterol consumption affect the lipid profile in plasma, as well as fat tissue deposition **(40).**

The gene expression and western blot of HMG-CoA reductase, the key enzyme of cholesterol synthesis, suppressed significantly in the edible oil fed rabbits as compared with the control rabbits, which suggested that the hepatic cholesterol biosynthesis was inhibited by the high dietary cholesterol. A higher expression of HMG-CoA reductase mRNA is considered to contribute to a higher serum cholesterol level related to increased levels of cytokines observed. Consumption of edible oil increased levels of liver lipids and also triggers Glucose-6-phosphate dehydrogenase (G6PDH) levels. It is suggested to facilitated lipogenesis, therefore G6PDH provides NADPH for palmitate formulation by fatty acid synthase **(42)** and for mevalonate generation by HMG-CoA reductase **(43).** It has been commonly considered that hyperlipidemia due to irregular lipogenic condition is one of the key predispositional factors for atherosclerosis in metabolic syndrome, in addition to hypertension **(44, 45).** G6PD plays a central role in the maintenance of the redox balance by NADPH-dependent enzymes, since this rate-limiting enzyme in the pentose phosphate pathway serves as the major provider of intracellular NADPH; G6PD provides NADPH for maintaining reduced glutathione in the recycling of oxidized glutathione to glutathione and for nitric oxide synthesis **(38).** Activities of anti-oxidative enzymes may be elevated in compensation for the increased oxidative stress. Thus, damages the activation of anti-oxidative enzymes, reflecting an enhanced reactive oxygen species, is a possibility if the vascular lesions were due to excessive reactive oxygen species generated via NADPH oxidase. In Group-II and group-III rabbits, overexpression of G6PD stimulates oxidative stress via NF-kB signaling activation, induces dysregulation of adipocytokines and promotes macrophage recruitment (40). Such an increase in oxidative stress by G6PD is paradoxical, because G6PD has also been thought to be an anti-oxidative enzyme **(46).** This suggests that the increased NADPH production by G6PD possibly results in an enhanced production of superoxide anion. In addition to the facilitation of lipogenesis, an increase in oxidative stress by G6PD may play a role in the more severe lesions in Group-III rabbit liver.

5. Conclusion:

In summary, it was confirmed in the present study that a 21days ingestion of edible oil causes a hyperlipidemic condition that may aggravate the liver and serum toxicity proper to Group III rabbits. Although a concomitant increase in HMGCR and G6PDH activity was observed, there was decreased in the activity of the anti-oxidative enzymes, superoxide dismutase, catalase in Group-II and Group-III rabbits. Thus, the increased activity of pro inflammatory cytokines IL-6, TNF – α and decreased activity of anti - inflammatory cytokine IL-10 appeared due to compensation for oxidative stress.

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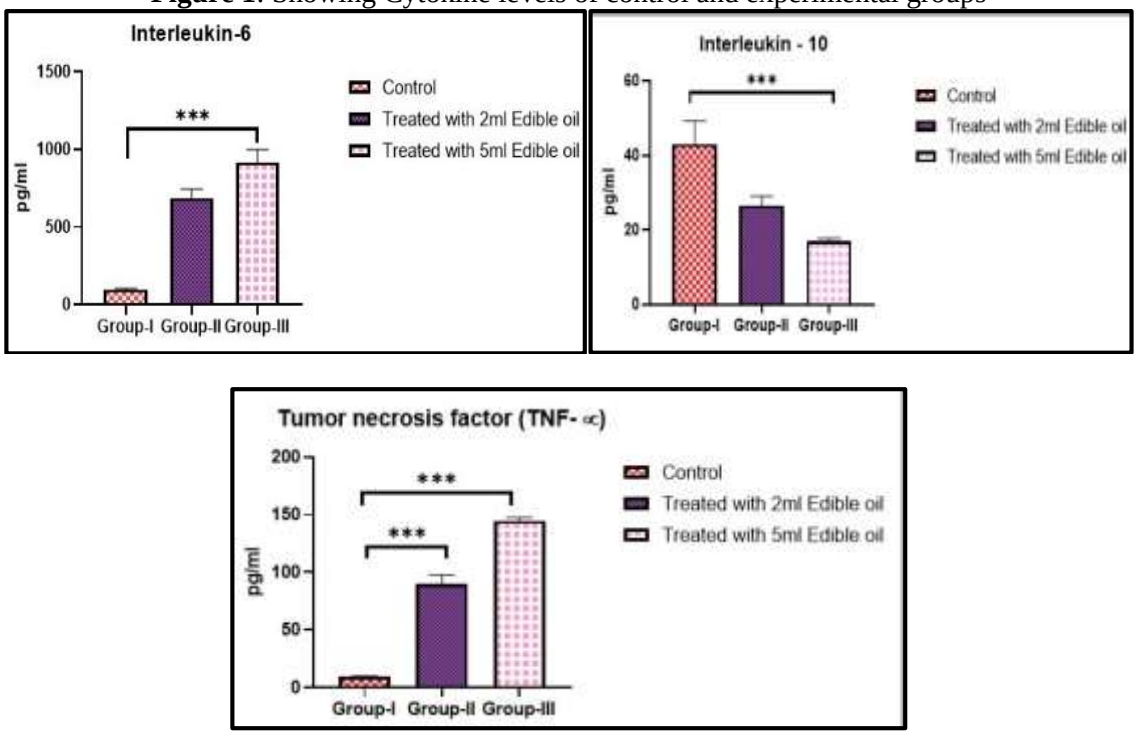
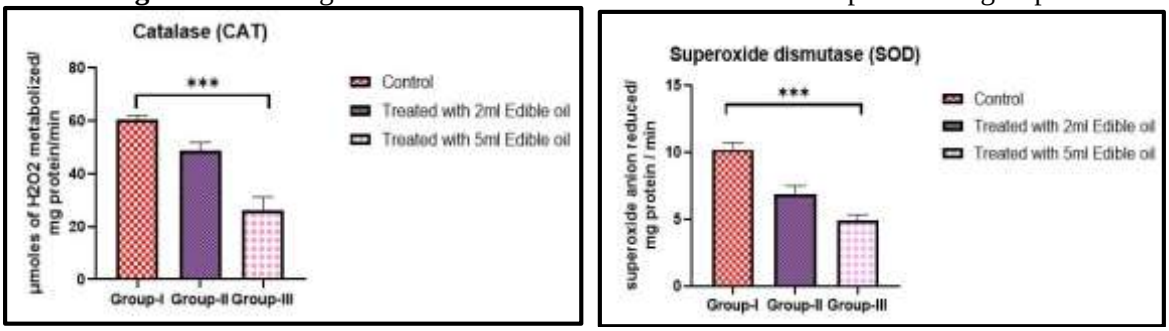
Figure 1: Showing Cytokine levels of control and experimental groups



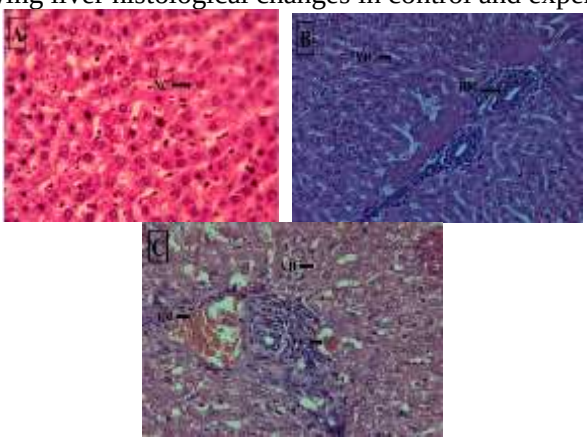
All values are expressed in Mean, ± SD (n=6), the values were analyzed with ANOVA
**** = p< 0.001 Group – I : Control rabbits ; Group - II : Treated with 2ml edible oil + Normal diet; Group – III : Treated with 5ml edible oil + Normal diet.

Figure 2: Showing Serum Antioxidant levels of control and experimental groups



All values are expressed in Mean, ± SD (n=6), the values were analyzed with ANOVA **** = p< 0.0001 Group – I : Control rabbits ; Group - II : Treated with 2ml edible oil + Normal diet; Group – III : Treated with 5ml edible oil + Normal diet.

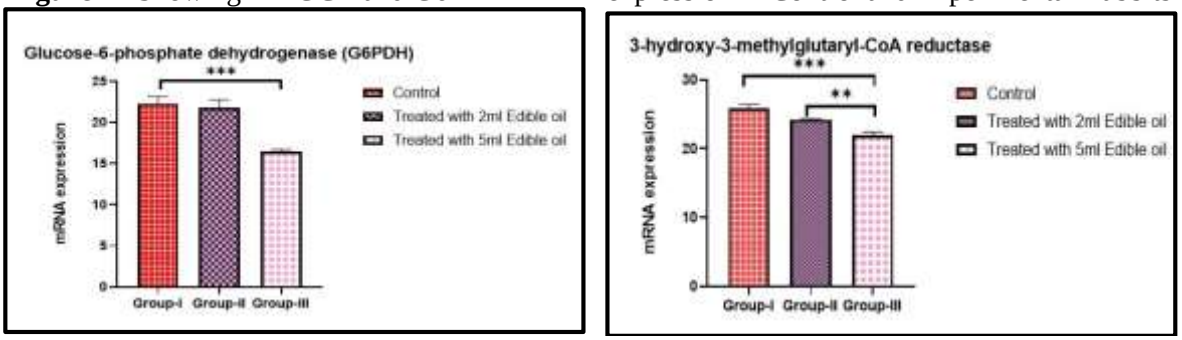
Figure 3: Showing liver histological changes in control and experimental groups



A) Normal hepatic cells of Liver tissue in control rats Normal cells (NC) (10X magnification). **B)** Shows mild hemarages (HM) and congestion, vacuolar degeneration (VD) leading to structural of liver treated with

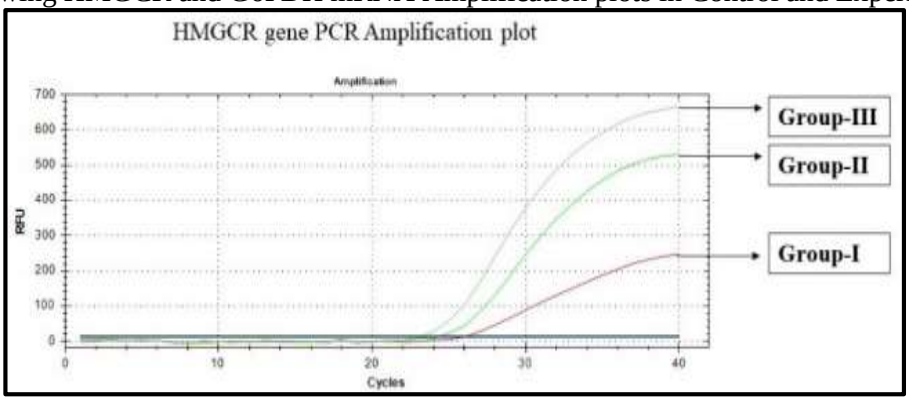
edible oil 2ml/Kg (10X magnification). **C)** Shows sever vacuolar degeneration, hemarages and portal vein (PV) leading to hepatic cells of liver treated with edible oil 5ml/Kg (10X magnification).

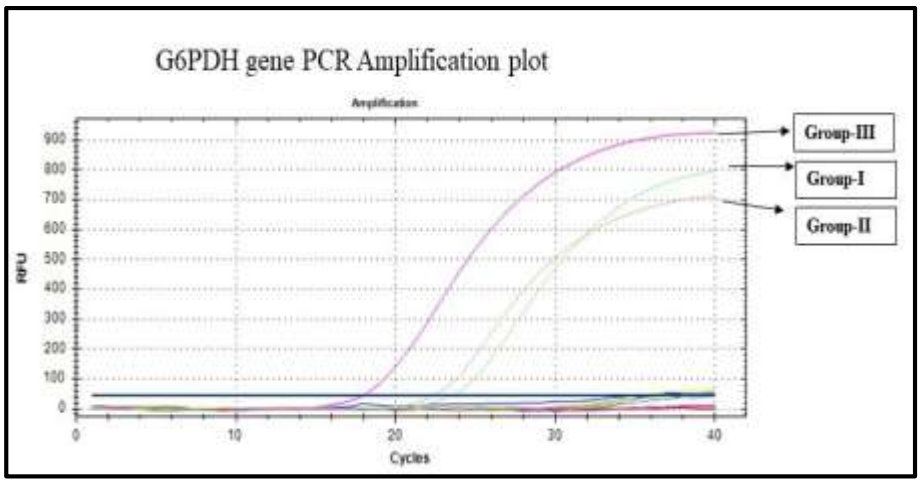
Figure 4 Showing HMGCR and G6PDH mRNA expression in Control and Experimental Rabbits



All values are expressed in Mean, ± SD (n=6), the values were analyzed with ANOVA **= p< 0.001; *** = p< 0.0001 Group – I : Control rabbits ; Group - II : Treated with 2ml edible oil + Normal diet; Group – III : Treated with 5ml edible oil + Normal diet.

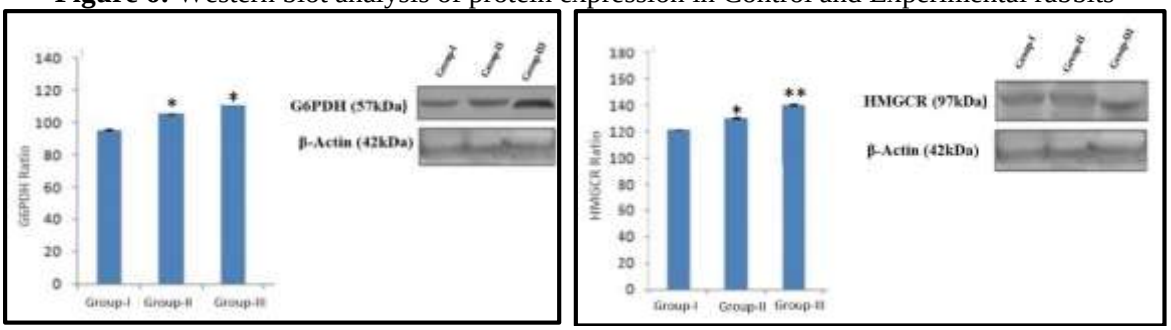
Figure 5: Showing HMGCR and G6PDH mRNA Amplification plots in Control and Experimental rabbits





All values are expressed in Ct Mean ± SD (n=6), the values were analyzed with ANOVA *= p< 0.05; **** = p< 0.0001 Group – I : Control rabbits ; Group - II : Treated with 2ml edible oil + Normal diet; Group – III : Treated with 5ml edible oil + Normal diet.

Figure 6: Western blot analysis of protein expression in Control and Experimental rabbits



All values are expressed in Western blot analysis of proteins G6PDH and HMGCR, mean ± SD (n=6), the values were analyzed with ANOVA *= $p< 0.05$; ** = $p< 0.001$ Group – I : Control rabbits ; Group - II : treated with 2ml edible oil + Normal diet; Group – III : treated with 5ml edible oil + Normal diet.