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BIOCHEMICAL EFFECTS OF RE-USED DEEP-FRYING VEGETABLE OIL IN CO-TREATMENT WITH VITAMIN E IN MICE

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Abstract: **Repeated heating of vegetable oil for different rounds of food frying is a common cooking practice that seems economical but the practice facilitates the oxidative degradation of lipids into reactive oxygen species which depletes natural antioxidants in food. Therefore, the biochemical effects of reused deep-frying vegetable oil in co-treatment with vitamin E in mice were evaluated. Twenty (20) healthy mice were randomly selected into 5 groups. Group 1, 2 and 3 were given 10ml/kgbw of water, vegetable oil (VO) and reused vegetable oil (reused VO) respectively. While group 4 and 5 administered reused VO and 210µg/kgbw Vitamin E (Vit E-210) and reused VO with 420µg/kgbw of Vitamin E (Vit E-420) respectively. Treatments was done once/day for 2 weeks. Standard analytical methods were used for analysis of selected biochemical parameters. The peroxide values analysis of the reused VO sampled ranged between 18-31mEq/kg. Treatment with Vit E-410 caused significant ($P<0.05$) reduction in urea level while both doses of Vitamin E (Vit E-210 and 420) caused significant ($P<0.05$) dose-dependent reduction in creatinine, ALT, AST, TG, total cholesterol, LDL and HDL when all were compared to reused VO. This study demonstrates that Vitamin E can ameliorate biochemical alterations caused by reused vegetable oil consumption.**

Keywords: **AST, Deep-frying, Kidney, Lipids, Liver, LDH, Oil, Peroxide, Vitamin E.**

I. INTRODUCTION

Using cooking oil more than once in food preparation, particularly for deep-frying, is a typical way to reduce expenses. Heating oil multiple times speeds up the breakdown of lipids, leading to the production of harmful reactive oxygen species and reducing the natural antioxidants present in the cooking oil. Research has indicated that vegetable oils are typically used for frying between three to six times before they are thrown away [1]. When frying oil is heated to a high temperature, it produces hydroperoxides and aldehydes. These harmful substances are taken up by food and subsequently absorbed into the gastrointestinal tract, eventually entering the bloodstream after consumption [2]. [3] stated that consuming frying oil that has been heated multiple times is linked to a higher risk of developing hypertension. Reusing frying oil can result in health risks, including changes in tissue structure and alterations in genetic material [4], [5]. Free radicals produced during frying can harm membrane lipids by causing lipid peroxidation, which may result in oxidative stress [6].

Food fortification can be applied to enhance the nutritional content of recycled cooking oil that has lost its nutrients. Food fortification involves adding certain vitamins and minerals to commonly consumed foods to help prevent or correct nutrient deficiencies. Vitamin E, particularly the isoform alpha-tocopherol, is an important antioxidant. Its primary function is to safeguard lipids from oxidative damage [7]. Vitamin E is a fat-soluble antioxidant that plays a significant role in preventing lipid peroxidation, which can cause damage to cell membranes, proteins, and DNA in the human body [8]. Vitamin E slows down the oxidation process by binding to free fatty acid radicals. However, it can only partially prevent the autoxidation of lipids and cannot reverse the creation of peroxides. Vitamin E is thought to help prevent chronic diseases, particularly cancer and cardiovascular diseases, because it acts as a scavenger of free radicals. There is a strong demand for this as an additional

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nutrient [9]. Therefore, this research assessed the biochemical effects of administering vitamin E along with reused vegetable oil in mice.

II. MATERIALS AND METHOD

A. Collection and Preparation of Sample

Reused deep-frying vegetable oil was randomly obtained from Five (5) different bean cake seller on the street of Afikpo, Ebonyi State, Nigeria. The fresh vegetable oil was also purchased in the supermarket. All were stored and preserved till when needed for analysis. Vitamin E supplement was obtained from a Pharmaceutical store in Afikpo, and doses were reconstituted based on their weight and reported recommended doses from literatures. Exactly 100ml of each of the five reused VO samples obtained from different vendors were measured and mixed together. The mixture (Reused VO) was used as representative sample for the whole reused vegetable oil and was given at 10ml/kgbw to mice orally but prior to the mixture the peroxide values of all oil were determined to ascertain their complete oxidation.

B. Peroxide values of reused and fresh vegetable oil

The peroxide values (PV) of the reused and fresh vegetable oil were determined by the standard methods outlined in [10]. Exactly 0.1087 ml of reused or fresh vegetable oil, which is equal to 100 mg of fat, was added to a conical flask and dissolved in 10 ml of glacial acetic acid. One ml of saturated potassium iodide solution was added to the mixture and allowed to sit for 2 minutes, with occasional shaking. A total of 20 ml of distilled water and 1 ml of starch solution were added. The content was titrated right away with 0.01N sodium thiosulfate until the endpoint was reached. The peroxide value was determined using the formula provided.

$$\text{PV (mEq peroxide/kg of fat)} = \frac{\text{Titre} \times \text{N} \times 100}{\text{Weight of sample}}$$

where

S = ml of $Na_2S_2O_3$ used (Test value– Blank value)

N = Normality of $Na_2S_2O_3$

W = Weight of fat in gram

C. Experimental Design

The experimental design consisted of 20 healthy albino mice divided into five (5) Groups with four mice per group. Groups 1, 2 and 3 were given 10ml/kgbw of water only, vegetable oil (VO) and reused vegetable oil (reused VO) respectively. while Groups 4 and 5 were co-administered with 210ug/kgbw of Vitamin E (Reused VO+ Vit E-210) and reused vegetable oil and 420ug/kgbw of Vitamin E (Reused VO+Vit E-420) and reused vegetable oil respectively. These treatments administered for once per day lasted for 2 weeks.

D. Biochemical Analysis

The animals were sacrificed and the animals were sacrificed and the serum was obtained for analysis. The Kidney (Creatinine, Urea) function was analysed using Randox kits (Randox Laboratories Ltd, UK). Liver function (Aspartate Aminotransferase (AST), Alanine Aminotransferase, [ALT]) according to methods of [11]. Lipid profiles (Total Cholesterol, Triglycerides, HDL and LDL) were done using colorimetric methods outlined in respective Randox reagent kits.

E. Statistical Analysis.

Graph pad Prism 8.2.0 software was used for analysis of the data obtained. One-way ANOVA was used for analysis, HSD Tukey Post Hoc Test and differences in the mean was taken to be significant at $P<0.05$.

III. RESULTS

In table 1, The average peroxide value for the reused vegetable oil is 22.57 mEq/kg, which shows a significant increase compared to the fresh oil. Four of the five measurements are greater than 19 mEq/kg, and one exceeds 31 mEq/kg. This shows that there is considerable primary oxidative breakdown in all the oils that have been reused. (Table 1.)

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Table 1. The mean peroxide value of fresh Vegetable oil and reused Vegetable oil

Vegetable Oils	Peroxide Value (mEq/Kg)
Fresh VO*	4.83 ± 1.87
Reused VO 1	20.60 ± 1.07
Reused VO 2	22.71 ± 2.5
Reused VO 3	19.82 ± 4.60
Reused VO 4	31.51 ± 5.20
Reused VO 5	18.23 ±3.40

In Fig. 1, Rats (Group 2) administered VO only up to Group 5 (reused VO+ 420mg/kgbw of Vit E) were significantly (P<0.05) lower in Urea concentration compared to groups that got water only. While Groups 2 and 5 were significantly (P<0.05) lower that group 2. But Groups 4 and 5 were significantly (P<0.05) different from group 3. (Fig. 1.) The creatinine concentration of Groups 2 to 5 was significantly (P<0.05) increased compared to Group 1. Groups 3 and 4 were significantly (P<0.05) increased compared to group in in terms of creatinine level. But both groups 4 and 5 were reduced significantly in creatinine level compared to group 3. (Fig. 1).

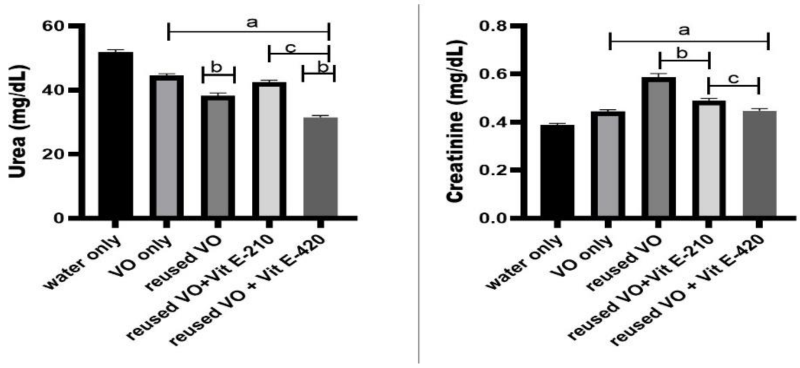

Fig. 1. Effects of Co-treatment of reused Vegetable Oil and Vitamin E on Kidney Function in Albino Rats. a = Significant difference vs water-only across the bracket. “b” = significant difference vs VO-only across the bracket. “c” = significant difference vs reused VO. ‘’d’ =significant difference vs reused VO+Vit E-210. Significant Difference is P<0.05 HSD Tukey post-test.

In Fig. 2. Rats (Group 2) administered VO only up to Group 5 (reused VO+ 420mg/kgbw of Vit E) were significantly (P<0.05) increased in ALT activity compared to groups that got water only. (Group 1). While Groups 3 to 5 increased significantly in in ALT activity when compared to Group 2. Groups 4 and 5 were reduced significantly (P<0.05). the AST activity in Groups 3 to 4 were significantly (P<0.05) increased compared to group 1 that received water only. Groups 3 to 5 were also significantly (P<0.05) different compared to group 2. While groups 4 and 5 were significantly reduced compared to group 3. (Fig. 2).

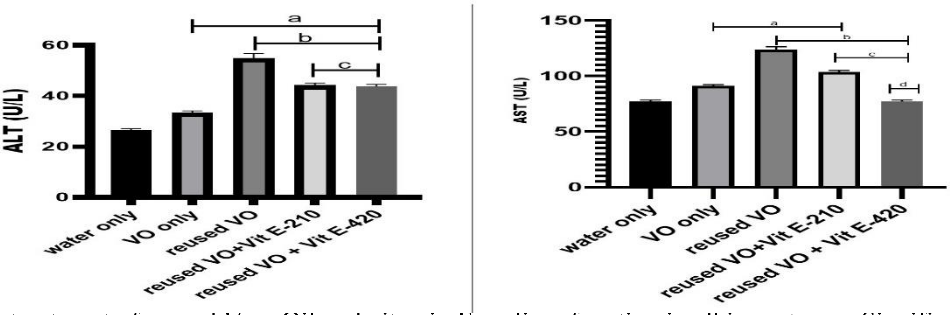

Fig. 2. Effects of co-treatment of reused Veg. Oil and vitamin E on liver function in albino rats. a = Significant difference vs water-only across the bracket. “b” = significant difference vs VO-only across the bracket. “c” = significant difference vs reused VO. ‘’d’ =significant difference vs reused VO+Vit E-210. Significant Difference is P<0.05 HSD Tukey post-test.

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In Fig. 3, triglycerides level of groups 2, 3 and 4 were significantly (P<0.05) increased compared to Group 3. Groups 3 to 5 were different significantly (P<0.05) compared to group 2. While groups 4 and 5 were lower than group 3 in TG level. (Figure 3). The total Cholesterol level of Groups 2, 3 and 4 were increase significantly (P<0.05) compared to group 4. There are significant differences between groups 3 and 5 compared to group 2. Also group 5 is significantly different compared to groups 3 and 4. (Figure 3.). The low-density lipoprotein (LDL) of groups 2 to 5 were significantly (P<0.05) different compared to group 1. Whereas, the levels of LDL reduced significantly in group 5 compared to groups 2,3 and 4. The high-density lipoprotein (HDL) levels of groups 2 to 5 were lowered significantly compared to group 1. Group 3 and 5 were significantly reduced in HDL compared to group 2 and then group 4 and 5 were lower significantly in HDL compared to group 3. Groups 5 was significantly compared to group 4.

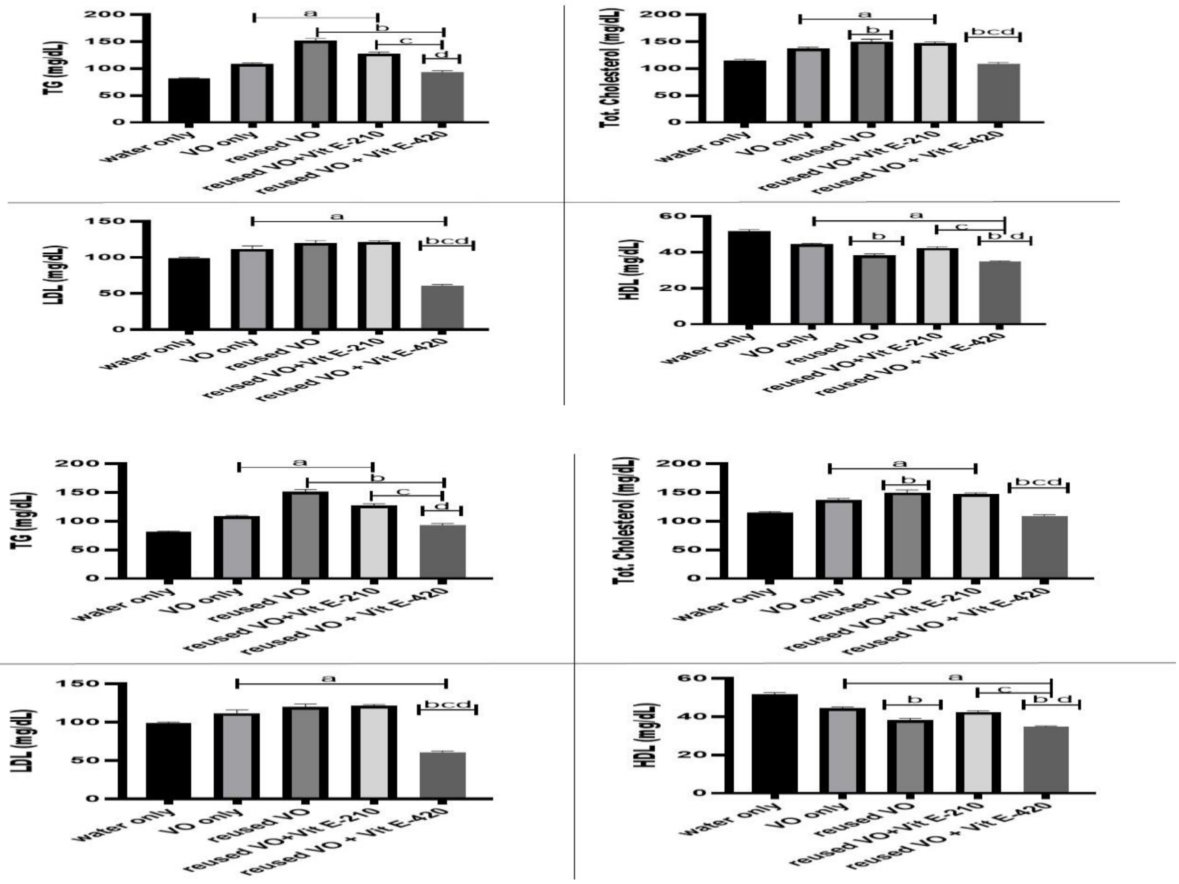

Figure 3. Effects of co-treatment of reused Veg. Oil and vitamin E on lipid profile in albino rats. a = Significant difference vs water-only across the bracket. "b" = significant difference vs VO-only across the bracket. "c" = significant difference vs reused VO. ''d' =significant difference vs reused VO+Vit E-210. Significant Difference is P<0.05 HSD Tukey post-test.

IV. DISCUSSION

The peroxide value of the fresh vegetable oil is low and consistent with typical levels found in fresh, minimally oxidized peanut oil. This aligns with the [12] guidelines for refined or edible oils, which generally suggest a value of less than 10 mEq/kg for acceptability. The peroxide value of the reused oil shows a notable level of primary oxidative degradation in all the reused oils. The differences are due to variations in how often oil is fried, how temperature is managed, the length of time oil is reused, methods of filtering oil, and the level of exposure to air and moisture among different vendors. Peroxide value assesses the amount of hydroperoxides, which are the main products of lipid oxidation that occur when oils are heated multiple times during frying. All reused samples exhibit higher levels of PV compared to fresh oil and often surpass typical regulatory limits for safe frying use. The highest value of 31.51 mEq/kg in Reused VO 4 indicates significant misuse, likely due to extended or repeated use. This raises concerns about the presence of harmful substances and a decrease in oil stability. This result aligns with the findings of [13] and [14].

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Reused vegetable oil negatively affects kidney function indicators when compared to the control group. It decreases blood urea levels and increases serum creatinine, suggesting changes in kidney function and potential harmful effects of reused oil on the kidneys. Supplementing with vitamin E while using reused vegetable oil can partially counteract these changes, and the effect is dependent on the dosage. This indicates that there may be a more intricate or dose-dependent impact on nitrogen metabolism, which could involve better clearance, changes in protein breakdown, or negative effects at higher doses. Research has indicated that a diet high in protein can increase blood urea levels [15]. [16] discovered that repeatedly heating cooking oil led to increased creatinine levels and a decrease in protein levels. The data indicate that reused VO negatively affects kidney function and that Vitamin E offers protective benefits.

Reused vegetable oil causes noticeable damage or stress to liver cells in this model, as indicated by significant increases in serum ALT and AST levels compared to water and fresh vegetable oil. Both ALT and AST levels increase, suggesting damage to liver cell membranes and/or mitochondrial injury due to oxidative or toxic effects from oxidized lipids produced during the repeated heating of oil. The co-treatment with vitamin E reduces these effects in a way that depends on the dosage. The 210 mg/kg dose partially lowers ALT and AST levels, while the 420 mg/kg dose leads to a more significant normalization, particularly for AST. This indicates that there is antioxidant protection against liver damage caused by reused vegetable oil. The observed pattern, which shows a significant increase with reused oil and partial to nearly complete normalization when vitamin E is present, suggests a process where products of lipid peroxidation in reused oil lead to oxidative stress and inflammation in the liver. Vitamin E, being a lipid-soluble antioxidant that interrupts the chain reaction of lipid peroxidation, helps prevent damage to cell membranes and reduces the leakage of enzymes. Research has shown that cooking oils that are heated or used multiple times can gather polar oxidation products, such as hydroperoxides, aldehydes, and polymerized triglycerides. These substances can lead to oxidative stress, inflammation, and damage to organs when ingested in animal studies. Increases in serum ALT and AST levels are often reported as indicators of liver injury [17], [18].

Using reused vegetable oil negatively affects plasma lipid levels compared to water-only or fresh vegetable oil. Specifically, triglycerides, total cholesterol, and LDL levels are higher, while HDL levels are lower when reused vegetable oil is consumed. Adding vitamin E to reused vegetable oil reduces some negative effects in a dose-dependent way. A higher dose of vitamin E lowers triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL) while increasing high-density lipoprotein (HDL) compared to reused vegetable oil on its own, although the levels are still typically worse than those found in water-only or fresh vegetable oil. This result is consistent with the findings of [17] and the review of [19]. Reused vegetable oil may cause damage through mechanisms such as oxidative products that can disrupt lipid metabolism or raise the secretion of hepatic VLDL. Heating the oil multiple times may produce oxidized lipids, which can increase cholesterol production or hinder its removal. Fried foods can produce oxidized lipids and trans fats, which may raise levels of atherogenic LDL cholesterol. Vitamin E, which is an antioxidant, helps to decrease oxidative stress and lower triglycerides. It may also reduce the oxidation of LDL cholesterol and improve the activity of LDL receptors or how the liver processes it. Additionally, antioxidants help maintain the amount and function of HDL cholesterol.

V. CONCLUSION

Reuse of vegetable oil caused impair kidney and liver function and promote dyslipidemia; antioxidant supplementation with vitamin E provides protection and mitigates the impairments in a dose-dependent manner. It reduces elevations in urea and creatinine, lowers ALT and AST relative to reused oil alone, and ameliorates the adverse changes in TG, total cholesterol, LDL, and HDL. This study demonstrates that Vitamin E can ameliorate biochemical dysfunction caused by reused vegetable oil consumption.

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