ORIGINAL ARTICLE

REUSED VEGETABLE OIL (RVO) CAUSED BRAIN LIPID PEROXIDATION AND A DECREASE IN ANTIOXIDANT MARKERS IN NORMAL ALBINO RATS

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ABSTRACT

Objective: Repeated use of vegetable cooking oils can lead to the oxidative breakdown of fats and oils, thereby releasing free radicals. This study investigated the impact of reused vegetable oil from fast food vendors in Abakaliki Metropolis on brain lipid peroxidation and antioxidant markers in normal albino rats. **Methodology:** We formed five (5) experimental groups (A-E) from a total of thirty-five (35) rats, each containing seven (7) albino Wistar rats. We collected the brain samples after 42 days of treatment for various laboratory analyses. **Results:** The amounts of catalase, superoxide dismutase (SOD), and reduced glutathione (GSH) were all significantly higher in rats that were given fresh vegetable oil (FVO) compared to rats that were given used vegetable oil (RVO). However, animals that had RVO had significantly greater levels of nitric oxide (NO) and malondialdehyde (MDA). In comparison to the control group, the albino rats that got RVO and FVO eventually had a noticeably larger body weight. **Conclusion:** The results suggest that reusing vegetable cooking oil can have a neurotoxic effect on the brain, and it is advisable to avoid it. According to the study's findings, consumption of reused vegetable oil could be harmful to the brain.

Keywords: Vegetable oil, antioxidant, lipid peroxidation, fast food.

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Conflict of Interest: None declared

INTRODUCTION

Deep-frying is a widely used and traditional method of meal preparation. It transfers mass as well as heat. To cut expenses, people frequently fried food in the same oil twice. Heating an oil repeatedly will modify its physical properties, making it thicker and darker in color (1), which can vary the number of fatty acids it contains. Heated oil passes through many chemical reactions, such as polymerization, hydrolysis, and oxidation (2). Many oxidative substances are produced during this process, including aldehydes and hydroperoxide, which can be consumed with the fried food (2).

Frying generates free radicals that can damage membrane lipids through lipid peroxidation, resulting in oxidative stress. Most people do not consider stress to be an illness in and of itself, even though there is a connection between prolonged stress and the development of many physiological and neurological problems (3, 4). Stressors are difficult stimuli that all living things must endure to sustain equilibrium (5).

The frying process exposes cooking oil to air and moisture, heating it to an unusually high temperature. In these conditions, a complex chain of chemical processes takes place, degrading the cooking. oil's quality and nutritional value. Most fast-food vendors in Abakaliki engage in the practice of reusing cooking oil due to its cost-effectiveness. Cooking oils heated repeatedly set off a series of events that eventually lead to the peroxidation of lipids and change the oil's fat content via hydrolysis, isomerization, polymerization, and oxidation (2). By oxidizing lipids, chemicals like free fatty acids, hydrocarbons, alcohols, ketones, epoxy, aldehydes, cyclic and trans-isomer compounds, and more are made. Some of these are volatile, while others are not. Therefore, excessive recycling of cooking oil intensifies foaming, darkens oil color, increases viscosity, and develops off-flavor. As a result of continual heating, cooking oil can degrade chemically and physically.

Numerous illnesses, including cancer, atherosclerosis, and neurological disorders, can be caused by oxidative stress-induced detrimental physiological processes. Oxidative stress contributes to aging through damage to the mitochondria (8). Fitó et al. (9) describe oxidative stress as an imbalance that benefits the body's oxidant system at the expense of the antioxidant system. Therefore, eating fast food made with recycled vegetable oil from Abakaliki, Nigeria may expose people to oxidative stress, the main factor behind the majority of illnesses.

The current study set out to examine the effects of recycling cooking oils made from vegetables. To assess food safety and quality during high-temperature processing or deep freezing, researchers

from Abakaliki Metropolis, Ebonyi State, Nigeria are looking at brain oxidative stress markers in a rat model. To determine if eating vegetable oils that have been recycled could hurt antioxidant levels and lipid peroxidation. This research, conducted in Nigeria's Abakaliki city, was the first of its type. Therefore, the purpose of this study was to ascertain how the antioxidant state of Wistar albino rats' brains was affected by their ingestion of recycled vegetable oil from fast-food vendors in Abakaliki Metropolis, Nigeria.

MATERIALS AND METHODS

Instruments and apparatus

The apparatus and instruments exhibit good analytical quality and grade.

Substances and agents

Chemicals and reagents of analytical grade were employed. The chemical suppliers in Darmstadt, Germany were BDH, Merck, and May and Baker. Our reagent sources were Biosystem Reagents and Instruments, Spain; Randox; QCA, USA; and commercial kits and products.

Biological Materials

This study used albino rats and fresh, reused vegetable oils.

The collection of biological materials

We purchased fresh vegetable oil from the International Market in Abakaliki, Ebonyi State, and collected reused oil from four different fast-food vendors in the Abakaliki metropolis. We purchased the albino rats from the veterinary facility, at the University of Nigeria, Nsukka, Enugu, Nigeria.

Methods

Experimental Animals

At the Ebonyi State University in Abakaliki, Nigeria, the rats were housed in stainless steel cages in an animal house facility with sufficient ventilation. The rats were brought up under ideal laboratory conditions, which included a 12-hour light/dark cycle and room temperature. The rats were fed regular rat food (Vital Feed®, Grand Cereals Ltd., Jos, Nigeria) and had unlimited access to water. We conducted our research according to the National Institutes of Health's standards for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, amended in 1996). The Ebonyi State University's Ethical Review Committee in Abakaliki, Nigeria, gave its approval for this work.

Experimental Design

Thirty-five albino Wistar rats were employed in this investigation. The rats were split up into five groups for the experiments as shown below.

Group A:	Rats were used as the normal control group, and they were fed and hydrated normally.

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Group B:	Rats received 5ml/kg body weight (b.w.) of FVO orally
Group C:	Rats were given oral doses of 5 ml/kg b. w. of RVO.
Group D:	Rats were given 2.5 ml/kg b. w. of RVO orally.
Group E:	Rats were given 1.5 ml/kg b. w. of RVO orally.

All the rats in the five groups received normal feed and water, and the trial lasted for 6 weeks. Rats' body weights were recorded at intervals of seven days. Rats were starved the night before being sedated with ether and sacrificed after receiving test compounds for 42 days. The brain was carefully removed, cleaned in ice-cold saline water, and dried. Following homogenization in 0.1 M phosphate-buffered saline (1:5 w/v, pH 6.4), the brain was centrifuged at 4000 x g for 20 minutes. GSH, MDA, and antioxidant enzyme activity were all examined using the produced supernatants.

Biochemical Analyses

Determination of Oxidative Stress Indices.

Determination of MDA

The Buege and Aust (10) method was used to determine the level of MDA in the sample. Two milliliters of the (1:1:1) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl, and 15% TCA), trichloro acetic acid-thiobarbituric acid-hydrochloric acid reagent, and one milliliter of the brain homogenate sample were combined. The mixture was then allowed to boil at 100°C for fifteen minutes before cooling. A centrifuge operating at 3,000 revolutions per minute was used for ten minutes to extract the flocculent materials. After the top layer was decanted, the absorbance at 532 nanometers was measured using a spectrophotometer against a blank.

Calculation of MDA: $\Delta A/min \times V_T/\Sigma \times V_S$

Where:

ΔA = changes in absorbance

V_T = totality of the volume

V_S = volume of the sample and Σ = molar extinction

Nitric Oxide

NO level was carried out by the method of Vogel (11). A ten-minute incubation period was provided at 37°C after fifty microliters of Griess reagent were added to fifty microliters of brain homogenate sample. The absorbance at 450 nm was measured using a microplate reader after 10 minutes.

SOD Activity

Fridovich and McCord (12) claimed that the suppression of adrenalin's antioxidant functioned as a stand-in for SOD activity. The reaction mixture was supplemented with 0.2 milliliters of the sample, 2.5 milliliters of 0.05 phosphate buffer (pH 7.8), and 0.3 milliliters of recently made adrenaline solution. This was quickly achieved in the cuvette using inversion mixing. Every 30 seconds, the rise in absorbance at 480 nm was recorded for a duration of three minutes, in comparison to a blank. Blank had 2.5 ml of buffer and 0.3 ml of adrenaline in it. The

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curve was used to calculate the SOD activity in the unknown sample, which was then represented as IU/mg protein.

Activity of CAT

The catalase activity was measured calorimetrically using the Sinha (13) method. The following were combined at room temperature: 1.0 milliliter of the sample, 4.0 milliliters of hydrogen peroxide (H2O2) solution, and 5.0 milliliters of phosphate buffer. The dichromate/acetic acid reagent contained 2.0 milliliters, to which one milliliter of the reaction mixture was added every minute. The stable absorbance value was then determined at 570 nm. Catalase activity (units per milligram of protein) = 8412 x A530 nm/min

GSH

The concentration of GSH was measured using Brehe and Burch's method (14) in 1976. 4.0% sulfur-salicylic acid was diluted in one ml of the sample, and the combination was centrifuged at 3,000 rpm for 15 minutes at 20°C. At 412 nm, the samples were added to 4.5 ml of Ellman reagent, and absorbance was calculated. While measuring absorbance at 412 nm, the blank was made by mixing 0.5 ml of 4% sulfur-salicylic acid with 4.5 ml of Ellman reagent. A similar treatment was given to a collection of standard solutions that contained 20 – 100 µg of GSH. The readings were given in milligrams per gram of protein.

Statistical Analysis

To analyze the data, a one-way ANOVA was performed using GraphPad Prism 8.0.2. Dunnett's Multiple Comparison Test was utilized to compare the groups after the fact. Mean ± SEM was used to represent the data. Differences were regarded as significant when the $p<0.05$.

RESULTS

Effects of Reused Vegetable Oil (RVO) on MDA and Antioxidant Markers

Rats that received RVO showed significantly lower activities of catalase, SOD, and level of GSH compared to groups that received fresh vegetable oil and normal control (Figures 3, 4, and 5). On the other hand, rats that received RVO showed significantly higher levels of MDA and NO (Figures 1 and 2).

PLACE FIGURES 1 - 5 HERE

FVO and RVO on Body Weight

There was an elevation in the body weight of the albino rats that received RVO and FVO when compared to the normal group in time time-dependent manner as shown in Figure 6.

PLACE FIGURE 6 HERE

DISCUSSION

Whether in a home or commercial environment,

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frying is still one of the most popular cooking techniques. The juicy flavor, outstanding flavor, brownish color, and crispy texture of fried food products are some of the organoleptic and sensory attributes that draw and appeal to consumers (15). Repeatedly heated cooking oil, thermal-oxidized cooking oil, and recycled cooking oil are commonly used interchangeably. Reusing this oil repeatedly has become commonplace due to a lack of public knowledge of its harmful effects (16). Recent increases in the consumption of deep-fried meals have the potential to increase the risk of obesity (17). Fried meals have a distinct and seductive quality that comes from the heat and mass transfer of food, oil, and air produced by deep-frying (18).

Rats that were given reused vegetable oil (RVO) showed reduced levels of catalase, SOD, and GSH in comparison to groups that were given fresh vegetable oil and a normal control group. Additionally, the rats that got RVO showed elevated levels of MDA and NO (Figures 1-5). The results of this study corroborated a study by Fan et al. (19) which demonstrated that when the number of frying cycles increased, the antioxidant activity of rice bran and palm oils dropped significantly. Fan et al. (19) reported that repeated heating reduced the stability of the rapeseed oil by increasing the amount of lipid peroxidation products and decreasing the amount of tocopherol. Similarly, it has been shown that after the twelfth frying process, deep-fat frying results in the total elimination of antioxidant activity (20). Adam et al. (21) found that rats' post-menopausal-induced lipid peroxidation was exacerbated by reheated soybean oil consumption. If the heating temperature and duration are increased, the antioxidant activity of vegetable oils may vary (2). Heat changes the oils' chemical and physical characteristics. Repeated heating causes the oil's quality to decline, leading to a decrease in the percentage of unsaturated fats and the emergence of more saturated molecules including trimers, dimers, monomers, high-molecular-weight compounds, and hydroperoxides. Lipid peroxidation may initially be stopped by antioxidants. On the other hand, oxidation—which results in rancid flavor and odor—is caused when vegetable oil is heated to a high temperature and then cooled again (22, 23). The oxidation process reduces the nutritional value and safety of food being fried due to the peroxidation of polyunsaturated fatty acids (PUFAs) (24, 25). Both non-enzymatic and enzyme-based antioxidants reduce ROS levels, which lessens oxidative cell damage. Enzymes like glutathione peroxidase, catalase, and SOD that directly neutralize ROS are considered to be the first line of defense (26, 27). On the other hand, non-enzymatic radical scavengers such as tocopherols, carotenoids, vitamin C, and phytochemicals such as phenolic compounds function as the second line of defense by

blocking the initiation of the oxidative cycle and halting its spread (28, 29).

As illustrated in Figure 6, the body weight results indicated a time-dependent, statistically significant rise in the albino rats who received RVO and FVO in comparison to the normal group. This outcome is consistent with the (30) report. This may be the result of reusing vegetable oil and causing fat to build up in the body's tissues.

CONCLUSION

Regularly consuming reheated vegetable oil can cause oxidative stress. Heating dietary vegetable oil frequently can lead to increased levels of malondialdehyde (MDA), reduced nitric oxide (NO), and diminished superoxide dismutase (SOD) and catalase activities. Additionally, it lowers levels of reduced glutathione (GSH). Repeated heating also decreases the oil's antioxidant capacity, worsening oxidative stress by increasing free radical accumulation and reducing antioxidant and vitamin levels. These oxidative effects significantly contribute to cardiovascular problems, which can be improved by dietary changes. Thus, limiting the consumption of repeatedly heated vegetable oil is crucial to prevent its harmful health effects.

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FIGURES

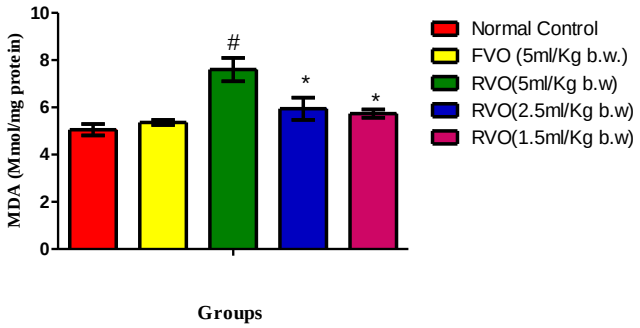


Figure 1: Effect of reused vegetable oil on Brain MDA Level in normal albino rats. The data is shown as mean ± S.D. (n=6). At $p<0.05$, mean values with varying signs revealed significant variations. FVO (Fresh vegetable oil) and RVO (Reused vegetable oil)

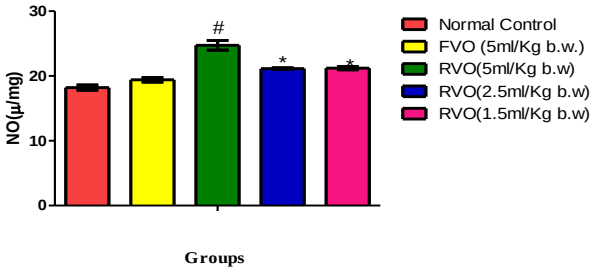


Figure 2: Effect of reused vegetable oil on Brain NO Level in normal albino rats. The data is shown as mean ± S.D. (n=6). At $p<0.05$, mean values with varying signs revealed significant variations. FVO (Fresh vegetable oil) and RVO (Reused vegetable oil).

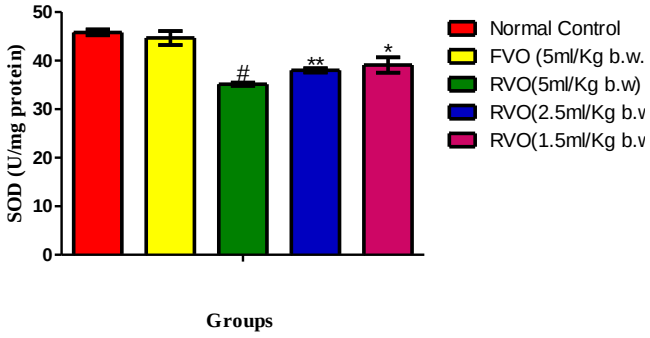


Figure 3: Effect of reused vegetable oil on Brain SOD Activity in normal albino rats. The data is shown as mean ± S.D. (n=6). At $p<0.05$, mean values with varying signs revealed significant variations. FVO (Fresh vegetable oil) and RVO (Reused vegetable oil)

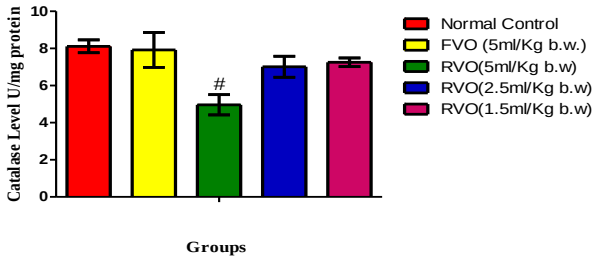


Figure 4: Effect of reused vegetable oil on Brain Catalase Activity in normal albino rats. The data is shown as mean ± S.D. (n=6). At $p<0.05$, mean values with varying signs revealed significant variations. FVO (Fresh vegetable oil) and RVO (Reused vegetable oil)

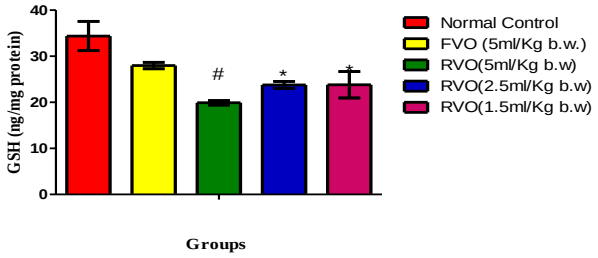
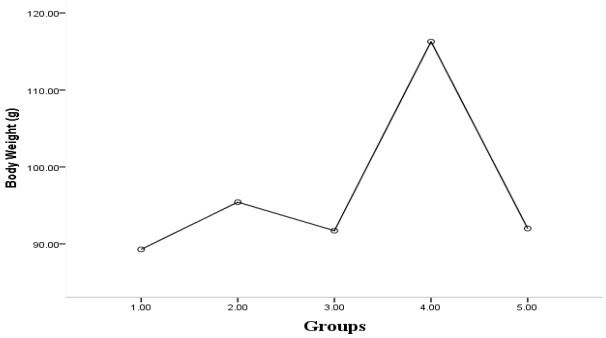


Figure 5: Effect of reused vegetable oil on Brain GSH Level in normal albino rats. The data is shown as mean ± S.D. (n=6). At $p<0.05$, mean values with varying signs revealed significant variations. FVO (Fresh vegetable oil) and RVO (Reused vegetable oil)



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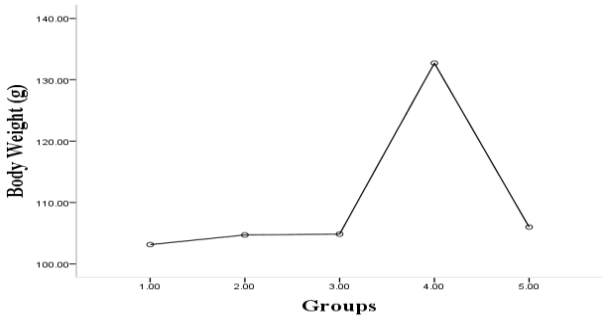

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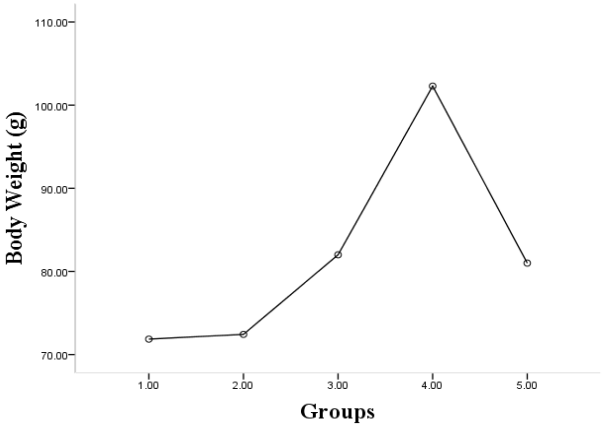

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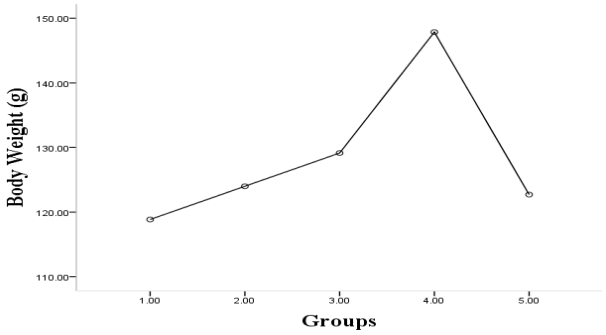

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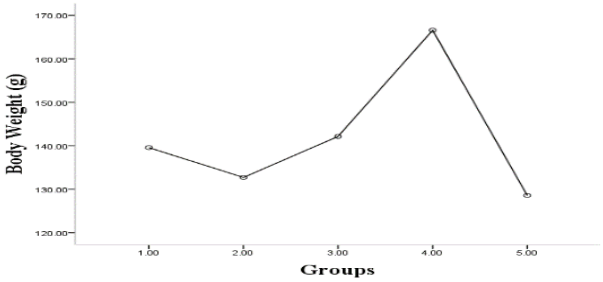

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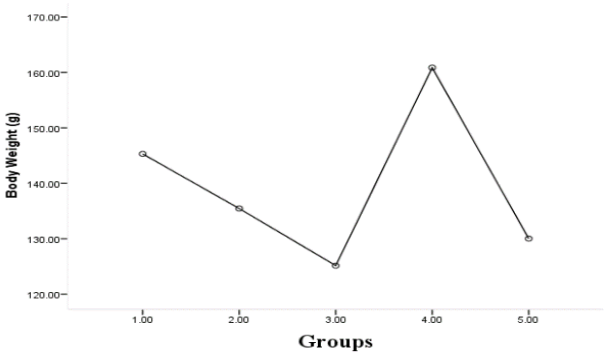

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Figure 6: Effect of Fresh and Reused Oil on Body Weight of Rats. Data are shown as mean ± S.D (n=6).

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