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Evaluation of the deleterious health effects of consumption of repeatedly heated vegetable oil

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ABSTRACT

Consumption of repeatedly heated cooking oil **(RHCO)** has been a regular practice without knowing the harmful effects of use. The present study is based on the hypothesis that, heating of edible oils to their boiling points results in the formation of free radicals that cause oxidative stress and induce damage at the cellular and molecular levels. Peroxide value of heated oil, histopathological alterations, antioxidant enzyme levels and blood biochemistry were determined in Wistar rats treated with the **RHCO**. **RHCO** revealed higher peroxide value in comparison to oil that has been unheated or singly heated. Histopathological observation depicted significant damage in jejunum, colon and liver of animals that received oil heated repeatedly for 3 times. The altered antioxidant status reflects an adaptive response to oxidative stress. Alteration in the levels of these enzymes might be due to the formation of reactive oxygen species (ROS) through auto oxidation or enzyme catalyzed oxidation of electrophilic components within **RHCO**. Analysis of blood samples revealed elevated levels of glucose, creatinine and cholesterol with declined levels of protein and albumin in repeatedly heated cooking oil group. Hematological parameters did not reveal any statistically significant difference between treated and control groups. Results of the present study confirm that the thermal oxidation of cooking oil generates free radicals and dietary consumption of such oil results in detrimental health effects.

1. Introduction

There were about 14.1 million cancer cases around the world in 2012. This number is expected to increase to 24 million by 2035. Colorectal cancer (CRC) is the third most commonly diagnosed and leading cause of cancer deaths in both men and women. Decades of expensive and replicating research has little impact on primary prevention of CRC. Sporadic cancer incidence attributes to 6% rate while environment and lifestyle constitute two third cases of CRC incidence (http://www.wcrf.org). Increased risk is attributed to factors like obesity, physical inactivity, alcohol consumption, long-term smoking, increased consumption of meat and fat rich food and low intake of fruits and vegetables [23]. Though several environmental chemicals have been implicated as the contributing factors to CRC in humans, one group of chemicals, the polycyclic aromatic hydrocarbons (PAHs) have generated the most interest as they are formed during cooking at high temperatures [41]. They are a single large family of compounds that have the potential to contribute significantly to dietary contamination, human intake and development of gastrointestinal tract (GIT) cancers [9]. Epidemiological studies have shown that diet contributes to 80% of the known CRC cases [6]. Foods like vegetables, fruits, oils, dairy products and meat are more prone to contamination with PAH during processing of food, cooking methods, time, temperature, amount of fat/oil added [38]. In this context, understanding the role of chemicals that are generated in food stuffs during its preparation towards the development of GIT cancers is important. Several studies on formation of mutagens during preparation of food have been done earlier but most of them were on items/cooking methods that are most often in practice in developed countries.

In India, consumption of fried foods made in road side eateries, food outlets in markets and restaurants is quite common. Socioeconomic status of people determines their food intake pattern. For example, in India people from low income group subsist on fried foods in roadside stalls. It has been reported in a survey that 48% of people consumed fried food 1–6 times/week [7]. Snacks account for 21% of all meals with the major types of snacks consumed constituting shallow and deep-fried foods [8]. Repeated heating of oils at

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high temperatures (160–190 °C) over a long period of time predisposes the oil to thermal oxidation, hydrolysis and polymerization with a configuration change of fatty acid from cis to trans isomers and accelerates the formation of oxidized and polymerized lipid species in the frying medium [18]. Repeated heating changes the physical appearance of the oil with increase in its viscosity, darkening in color, foaming and decrease in smoke point making it harmful for human consumption.

Few studies in India evaluated the genotoxic potential of such heated oils [49,50] but to our knowledge, no attempt has been made till date to evaluate the harmful consequences of the usage of repeatedly heated cooking oil (**RHCO**) in Hyderabad, in particular. Several investigations that have been carried out in animals demonstrate that consumption of **RHCO** increases the presence of reactive oxygen species (ROS) and thus a decreased radical scavenging activity and thereby oxidative stress [17]. Use of **RHCO** is known to induce genotoxicity [10] and there by carcinogenicity [45]. Deleterious health effects of consumption of **RHCO** like increased blood pressure [11,40,18,19], risk of cardiovascular diseases [24,26–28], endothelial dysfunction [21], impaired vasorelaxation responses [33], hypertension [48], increased lipid peroxidation and LDL [12,46] and atherosclerosis [1] are available in literature. Several investigations in rats also revealed functional changes in blood vessels, changes in serum alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase levels [34]; intestinal damage and impaired function, mal-absorption of glucose [30]; impaired kidney function with increased blood pressure [32]. In contrast few studies reported no significant damage induced by use of **RHCO** in animals [44]. In vitro cytotoxicity assays in Hep G2 cell lines suggested that extract of fish oil that has been repeatedly boiled and heated for frying has substantial cytotoxic potential [36]. In contrast, samples of six cooking oils with different levels of unsaturation both heated and unheated did not show any mutagenicity with Ames test, with or without metabolic activation [51].

The objective of a study from Kuala Lumpur was to determine the level of knowledge, attitude and practice of night market food outlet operators regarding the usage of **RHCO**. The data collected from the 100 by face-to-face interview using a questionnaire showed that 67.0% agreed this to be not a good practice, 69.0% agreed that the use of **RHCO** is detrimental to health and 63% admitted that they had used **RHCO** [4]. In view of the information from various investigations done earlier, the present study has been designed to investigate the harmful consequences of consumption of **RHCO** (here vegetable oil) in Wistar rats. Peroxide value is a useful method to determine the quality of oil. It is an index to measure the concentration of hydroperoxide, which is formed during lipid oxidation [13]. Since there is dearth of information on the oxidative stress induced pathogenesis, antioxidant status was assessed in the animals by assessment of levels of radical scavenging enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and rate of lipid peroxidation (LPO). This gives a measure of exposure induced oxidative stress that results in inflammation and damage to macromolecules including DNA, proteins and lipids [5]. Further, exposure dependent changes (if any) in blood biochemistry was estimated in the present investigation by determination of glucose (GLU), cholesterol (CHOL), creatinine (CRE), protein (PRO) and albumin (ALB). The evaluation of hematological parameters like total red blood cell count (RBC) and white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), hemoglobin (Hb) and hematocrit gives the profile of perturbations in blood if any, following consumption of **RHCO**. The present investigation is thus based on the hypothesis that consumption of RHCO contributes to intestinal tumor development through altered biotransformation and generation of free radicals that in turn induce damage at cellular and molecular level.

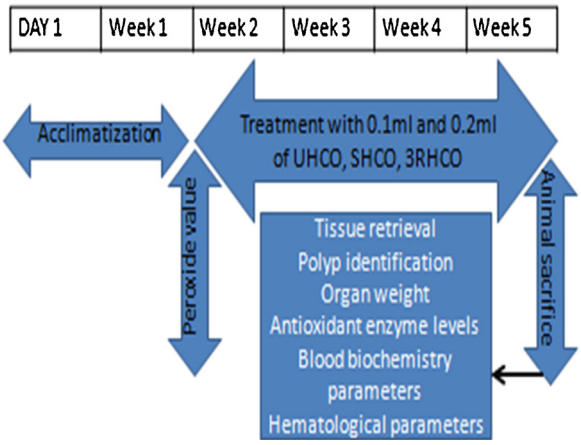

Fig. 1. Schematic representation of experimental design.

2. Materials and methods

2.1. Preparation of oil sample for treatment

The refined vegetable cooking oil (5 L) of a standard food grade was purchased from the local market. An aliquot of oil (1 L) was separated and labeled as unheated cooking oil (UHCO). Another aliquot of oil (4 L) was heated (above 300 °C) above its smoke point for 30 min and then cooled to room temperature. From this, a sample of 1L was separated and labeled as singly heated cooking oil (SHCO). The same process was repeated to obtain oil heated 3 times (3RHCO). This process of heating and cooling of the oil was performed without addition of fresh oil. The viscous dark brown oil sample (approximately 2L) thus obtained was then stored in amber color bottles to prevent photodegradation of PAHs.

2.2. Analysis of constituents of oil

The quality of oil used in this study for treatment was investigated by determination of its peroxide value (PV) using standard titration method by American Oil Chemists' Society (AOCS). This method determines all components, generally assumed to be peroxides or other similar products of fatty acid oxidation. PV is expressed in terms of milliequivalents of peroxide per 1000 g of test sample that oxidizes potassium iodide under test conditions ($mEqO_2/kg$) (Fig. 1).

2.3. Animal treatment

Institutional animal ethical committee approval was taken prior to the beginning of the study. Twenty eight day repeated oral dose toxicity study was performed using male and female rats based on OECD guideline 407 [2008]. Wistar rats of 6–8 week age and weighing approximately 80–120 g were purchased from the research animal suppliers in India (National Institute of Nutrition, Hyderabad). Rats (n = 10; 5 male and 5 female in each treatment group) were then kept in laboratory grade polycarbonate cages and housed in institutional animal care facility to ensure humane care and use of laboratory animals. All animals were allowed a seven-day acclimation period prior to being randomly assigned to the following treatment categories: (i) Unexposed control rats (diet only) (ii) rats treated with unheated cooking oil (UHCO) (iii) rats treated with singly heated cooking oil (SHCO) (iv) rats treated with repeatedly heated cooking oil (3RHCO). Animals were administered 0.10 mL and 0.20 mL of oils of above heated grades via oral gavage for 28 days. The test animals were observed for symptoms and mortality each day during the treatment period. Body weight of animals