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Evaluation of Effect of Repeated Heated Sesame Oil on Hematology and Vital Organs of Wistar Male Rats

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Abstract: ***Sesame oil (SO) is known for the natural antioxidants present in it and their advantages. Upon thermal treatment the activity of antioxidants varies and toxicity may be developed in the oils. The purpose of this study is to know the toxic behavior of repeatedly heat treated sesame oil with and without synthetic antioxidant, tertiary butyl hydroquinone (TBHQ) on Wistar rats. SO with and without synthetic antioxidants was heated at 180 ± 10^0 C for 16 hrs and physico - chemical changes of the oils were studied. The effects of repeatedly heated oil on the vital organs of wistar male rats were investigated. The ground standard rat chows (Gold Coin Sdn Bhd, Malaysia) were mixed with thermal treated SO and fed to the rats for 30 days. Twenty - four male wistar rats were divided into four groups, control rats (CR), fresh SO (SOR), repeated heated SO (HSOR) and repeated heated SO containing 200 ppm TBHQ (HSOQR) fed rats. After one month, the rats were sacrificed and hematological tests of the heart, kidney and liver tissues were carried out along with histological study. It is found that, in SOR groups, there were no significant variations in hematology tests and damage in the heart, kidney and liver tissues, whereas the damage were found to be more significant in HSOR and HSOQR rat groups. Also the deleterious effects were more in rats consuming repeatedly heated SO without synthetic antioxidant.***

Keywords: Wistar male rats · Sesame oil · Repeatedly heated oil · TBHQ · Hematology

1. Introduction

Deep frying is a popular method of preparing food and snacks (1). During deep frying process, the cooking oil is heated at high temperature with exposure to air and moisture, resulting in lipid peroxidation (2, 3). This thermal deterioration generates harmful oxygen reactive species which might be deleterious to the cardiovascular system. However, reusing the same cooking oil for deep frying before discarding, is common in the society, not only by road side vendors but in household as well (4). It reduces the cost of food preparation; such practice may cause exposure to harmful oxidative compounds.

The main uses of reused oils at present are in animal feed and in a much smaller proportion in the manufacture of soaps and biodegradable lubricants. As a consequence, the use of recycled cooking oils in animal feed must be studied from the point of view of safety as the fats and oils which are heated at high temperature during deep frying which may generates high levels of cytotoxic products. It may promote the initiation, development and progression of cardiovascular diseases (5). Peroxidation of biological systems is regarded to be associated with a number of pathological manifestations. Effects of various types of dietary fats including vegetable oils on plasma lipid and lipoprotein concentrations and the influence of their constituents fatty acids on heart disease have been reported (6).

Much research evidence suggests that the protective role of fresh sesame oil upon prolong feeding against oxidative stress is through its antioxidant mechanism (7 - 9). It has been identified that presence of natural antioxidants in sesame oil like sesamin, sesamol and sesaminol might be a responsible components for its protective action (10, 11). However, at frying temperature, sesamin and sesamol will decompose and will not give any antioxidant effect. There will be only some effect of antioxidant sesaminol (12 - 14) at frying temperature for few hours. At the same time there will be presence of decomposed products of triglycerides which are harmful.

Among the synthetic antioxidants, tertiary butyl hydroquinone (TBHQ) has been reported to be more effective than other regular synthetic antioxidants such as butylated hydroxyl aniline (BHA) and butylated hydroxyl toluene (BHT) (15 - 17). Also systematic study made by Allam and Mohammed 2002 reported that TBHQ is more stable.

In the recent years the effect of heated oils on rats have been studied by few scientist mainly on palm oil (18 - 21). Ng et al (2013) have reported long - term intake of food containing recycled deep - frying oil causes blood pressure elevation and vascular hypertrophy in rats. Adam (19) have reported that protective effect on the aorta may be lost when the palm oil is repeatedly heated. Leong et al (22) have reported repeatedly heated palm olein may have negative effect on the activity of blood pressure–regulating enzymes and increase lipid peroxidation. Very few scientists have investigated on heated sunflower oil effects on biological activities of rats. (23, 24). They have reported heated sunflower oil - induced hyperlipidemia. Further they have studied that the highly unsaturated sunflower oil was less resistant to the oxidative stress produced by frying and led to a higher degree of lipid peroxidation in liver microsomes. To best of our knowledge there is almost no information on effect of repeatedly heated or fried sesame oil (SO) on the vital organs of rats have been reported. Hence, authors in the present investigation have been studied the effect of repeatedly heated SO with and without antioxidant, TBHQ on the vital organs of rats by assessing the body weight of

animals, hematology and histopathological examination. Also, the structural changes in the triglycerides or fatty acids occur during heating, which leads to increase in the toxicity in oil have been studied using ^{1}H NMR.

2. Experimental

Materials and Methods

Heated oil and diet preparation

Sesame oil used in this study was obtained from M/s. Sri Murugan Oil Mills, Mandya, India. Tertiary butyl hydroquinone (TBHQ) was obtained from Sigma Aldrich and AR grade chemicals were used for other chemical analysis.

The repeatedly heated oils were prepared by heating sesame oil with and without TBHQ in a home fryer (Model No. SDF - 8503, 120V/230V ~ 1500W Chennai, India) at about 180 ± 10°C for 4 hr per day and repeated the same for 4 days. After each heating cycle, the oils were cooled and chemical analysis has been performed to know the level of thermal oxidation.

Physico - chemical Properties

Peroxide value (25)

Peroxide value (PV) was determined according to AOCS official method Cd 8 - 53

p - anisidine value (p - AV) (26)

It is a measurement of carbonyl content in the oils or fats, and was determined by the standard method according to AOCS.

Total oxidation value (TOTOX) (27)

TOTOX was used to estimate the oxidative deterioration of lipids. TOTOX value is used to indicate over all oxidation of oil. Using both values PV and p - AV, total oxidation was calculated using the formula as follows.

TOTOX value = 2 × PV + p - AV (1)

Free fatty acid

The FFA content, as the percentage of oleic acid, was determined as per AFNOR NF T 60 - 204 standard method.

Polar compounds (28)

The contents of total polar compounds of oils were determined as per the proposed method by IUPAC.

Study design of Animals

After the fourth cycle of repeatedly heating process, the sesame oil was used for rat feeding along with rat chow. Standard rat chow (Gold Coin, Kepong, Malaysia) was ground and formulated by mixing 15% (W/W) of SO, HSO and HSOQ. The mixture was reformed into pellets and dried in an oven at 80 °C overnight.

Twenty - four male wistar rats weighing (175 - 200 g) were grouped into 4 groups (six rats in each group) by random distribution and housed in individual cages, and kept at room temperature of 27˚C ± 2˚C under a 12 hr light/dark cycle, in an approved animal house facility at Defence Food Research Laboratory, Mysore. The experimental protocol was approved by the institutional animal ethics committee. After one week of adaptation, the rats were randomly and equally assigned to four groups (six rats in each group). The food formulations fed to rats for 30 days along with sample code is tabulated in Table 1.

Table 1: Different food formulations given to rats with fresh and heated sesame oil

Group	Food diet or formulations	Sample code
1	Rat chow only	CR
2	Rat chow mixed with 15% w/w fresh sesame oil (SO)	SOR
3	Rat chow mixed with 15% w/w repeated heated sesame oil (HSO)	HSOR
4	Rat chow mixed with 15% w/w heated sesame oil with 200 ppm of TBHQ (HSOQ)	HSOQR

Animals were given fresh diet *ad libitum* daily, the gain in body weight of animals was monitored at regular intervals of time. The animals had free access to food and water throughout the study. After 30 days of feeding, rats were fasted overnight and sacrificed under diethyl ether anesthesia. Before sacrifice, blood was drawn by cardiac puncture, and serum was separated by centrifuging at 2000 rpm for 20 min at 4 °C and hematological tests were performed.

Hematological analysis

An hematological autoanalyzer (Orphee Mythic 22 Hematological Analyzer; Diamond Diagnostic; USA) was used to determine different hematological parameters, such as red blood cells (RBC), white blood cells (WBCs), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), lymphocytes (LYM%), LYM count, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT and platelets count (PLTs), mean platelet volume (MPV) and platelet larger cell ratio (P - LCR).

Statistical analysis

All the hematological tests, change in body weight and organ weights were expressed as mean ± standard error of the mean of 6 rats per experimental group. The results were analyzed by analysis of variance (ANOVA). Analysis of variance was employed to evaluate the differences between the groups. A difference of $p < 0.05$ was considered to be significant.

All the physico - chemical analysis were carried out in triplicate and expressed as mean ± standard error of the mean and $p < 0.05$ was considered to be significant.

3. Results and Discussion

Table 1: Changes in the properties of sesame oil after each cycle of heat treatment at 180± 10 ^{0}C for different time intervals with and without TBHQ

Heating Time (hr)	Peroxide value (meq/kg of fat)		p - anisidine value (m mol kg $^{-1}$)		TOTOX value	
	HSO	HSOQ	HSO	HSOQ	HSO	HSOQ
0	2.62 ± 0.12	2.52 ±0.14	2.27 ±0.15	2.27 ± 0.15	7.51±0.35	7.51±0.34
4	10.78 ±0.16	5.32± 0.12	13.2 ± 0.46	8.03 ±0.50	34.76±0.56	18.67±0.86
8	18.89± 0.16	6.62 ±0.24	24.85±1.11	15.78±1.07	62.63±1.12	29.02±1.24
12	20.86 ±0.35	7.42 ±0.23	36.3 ±1.12	26.0 ±1.76	78.06±1.82	40.88±1.54
16	19.50±0.41	8.48 ±0.33	50.13±2.11	35.75±1.54	89.13±1.28	52.71±1.68

Values are Mean ± S. D (n = 3) at $p < 0.05$

Heating Time (hr)	Free fatty acid (%)		Total polar compounds (%)	
	HSO	HSOQ	HSO	HSOQ
0	0.11±0.01	0.10±0.01	4.1±0.11	4.15±0.12
4	0.14±0.02	0.12±0.03	7.6±0.21	6.2±0.23
8	0.18±0.02	0.15±0.02	11.5±0.13	7.9±0.22
12	0.23±0.01	0.17±0.01	15.8±0.31	9.1±0.28
16	0.31±0.03	0.18±0.03	18.5±0.14	10.5±0.25

Values are Mean ± S. D (n = 3) at $p < 0.05$

Free Fatty Acid Value

Formation of free fatty acids (FFA) during frying process is considered to be a measure of rancidity of oils. FFA content is used to probe the shelf life of fried oil, but it is not recommended to be the only indicator. The percentage of FFA formed during the heating process has been indicated in Table 1. After heating period the sesame oil showed a higher FFA values than the TBHQ loaded oils. FFA is formed due to hydrolysis of triglycerides and may get promoted by the reaction of oil with moisture (29). Addition of antioxidant caused significant reduction in FFA values of sesame oil.

Changes of polar compounds in oil

Generally, degradation of oil during frying is accompanied by increasing the polar compounds of oil (30). Many researchers reported that the total polar components (TPC) to be the most reliable indicator of oil degradation (31–33). Polar compounds include all oxidized triglycerides, dimerized triglycerides, FFAs, monoglycerides, diglycerides, sterols, antioxidants, antifoamers, hydrogenation catalyst residues and soaps (34). Table 1 shows the percentage of TPC formed during repeatedly heating sesame oil and sesame oil with 200 ppm of TBHQ. After 16 hrs of heating, the final TPC values were 18.5 % in SO without TBHQ and 10.5% in formulated oil. Polar compounds are sum of non triglycerides of oil including fatty acids, alkaline pollutions, sterols, tocoferols, mono and di triglycerides, alcohols, aldehydyes, ketones and other soluble compounds in oil that are more polar than triglycerides (35). According to few researchers (36, 37) the degradation of oils can be measured by formation of polar compounds which indicate the breakdown of triglycerides. Polar compounds accumulate on the surface of frying pan and food products during frying. It can be imagined that the most poisonous material are exist in the polar compound of oil (38).

Peroxide value

Hydro peroxides are the primary products of the oxidation of oils; therefore, determination of peroxides can be used as an oxidation index (OI) for the early stages of oil oxidation (39, 40) Hydroperoxides are odorless and colorless, but are labile species that can undergo both enzymatic and non - enzymatic degradation to produce a complex array of secondary products. Measuring the content of primary oxidation products is limited due to the transitory nature of peroxides, but their presence may indicate a potential for later formation of sensorial objectionable compounds. PV increases only when the rate of peroxides formation exceeds that of its destruction. Changes in the peroxide values of the sesame oil samples under investigation during the heating process are tabulated in Table 1 at the end of the fourth cycle. The peroxide value (PV) of SO without antioxidant was about 19.50 meq of oxygen/kg of fat and the SO mixed with 200 ppm of TBHQ was about 8.48 meq of oxygen/kg of fat respectively. These results were consistent with the data published elsewhere (41 – 44) and show that the antioxidant compound TBHQ have an important role in inhibiting of free radical formations during the initiation step of oxidation, interruption of the propagation of the free radical chain reaction by acting as an electron donor, or scavengers of free radicals in oil.

p - Anisidine Value (p - AV)

During lipid oxidation, hydroperoxides, the primary reaction products, decompose to produce secondary oxidation products (aliphatic aldehydes, ketones, alcohols, acids and hydrocarbons) which are more stable during the heating process, responsible for off - flavors and off - odors of edible oils. In order to ensure a better monitoring of lipid oxidation process in the heating time, the simultaneous detection of primary and secondary lipid oxidation products is necessary. p - AV is a reliable measurement of the amount of secondary oxidation products. Table 1 presents the changes recorded in p - AV in the heating time and as effected by supplementation with TBHQ. It can be observed that repeated heating promoted rapid transformation to secondary products which contributes to the off - flavors of SO. Addition of 200ppm of TBHQ resulted in significant decrease in p - AV ($p < 0.05$) relative to the control sample. These data are in agreement with those reported elsewhere (45, 46) which highlight that the natural extracts showed a significant inhibitory effect against thermal oxidation

At the end of heating, changes in p - AV was minimum for SO with 200 ppm TBHQ, which shows efficiency of antioxidant. The increase in p - AV of sesame oil with 200 ppm TBHQ has been reduced by 28% as compared to control oil.

Total Oxidation Value

The PV and p - AV data together provides a comprehensive overview of the oxidation process in oils. This is a mathematical prediction of oxidative stability (OI) and the value is calculated as TOTOX value. TOTOX value was

used as an indication of overall OI and was correlated with the extent of oil deterioration. TOTOX values for samples mixed with TBHQ are significantly lower than the value registered for control.

These parameters are indicators of the state of oil degradation and the results indicated that SO with 200 ppm of TBHQ was more stable chemically than SO without TBHQ.

Animal results

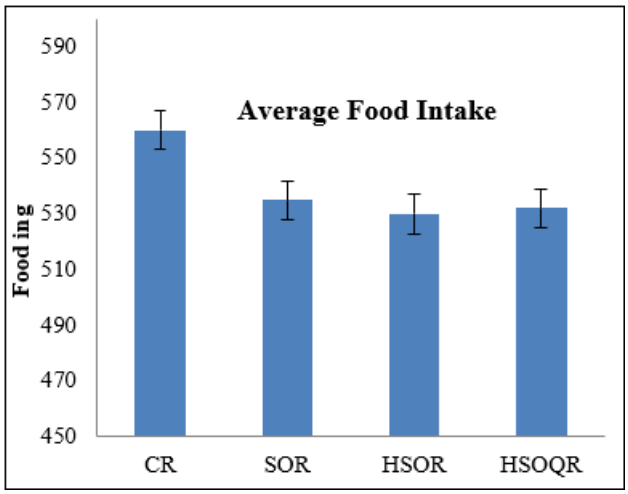

Figure 2: Average food intake by CR, SOR, HSOR and HQSOR

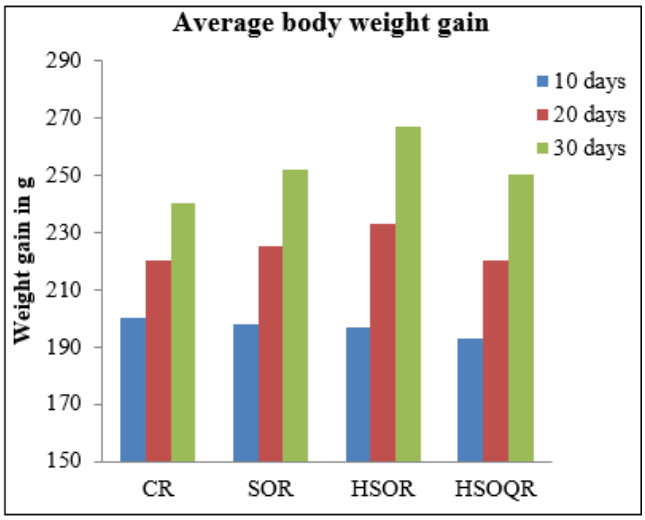

Figure 3: Average body weight gain by CR, SOR, HSOR and HQSOR at regular intervals of time (10 days)

Food intake and body weight

Fig.2 indicates the food intake by CR, SOR, HSOR and HSOQR groups. From the figure it was noticed that rats treated with sesame oil along with rat chow i. e., the SOR, HSOR and HSOQR groups showed average food intake lower compared to the CR groups. After 30 days of feeding, the CR, SOR and HSOQR groups had lower body weight gain compared to the HSOR groups as seen in Fig.3 ($p < 0.05$).

The addition of sesame oil, either in fresh or repeated heated forms, reduced the average food intake in experimental rats compared to food without SO. This reduction shows that the rats preferred normal rat chow diet without SO. It may be due to the addition of oil, which modified the taste and smell of the diet, may have contributed to the reduction of their food intake. The heating process, however, did not have any significant effect on their food intake. All sesame oil - treated groups showed a similar food intake; regardless of the 16 hr heating the oil (47). The HSOR group showed a significant body weight gain after 30 days of feeding as compared to the CR group. Despite their low food intake, the HSOR group exhibited the highest weight gain compared to the CR, SOR and HSOQR groups. It may be due to the weight gain in the HSOR and HSOQR group is caused by water retention. An earlier study reported that water retention may be due to multiple causes, such as renal failure or hypertension, which affected weight gain (48, 49).

Effect of food intake on organ weights

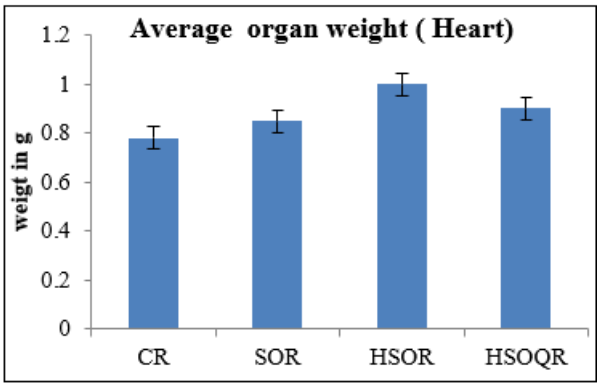

Figure 4: Average weight of heart of CR, SOR, HSOR and HQSOR groups

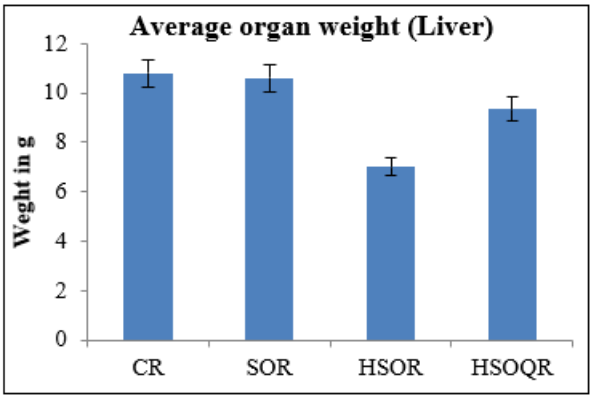

Figure 5: Average weight of liver of CR, SOR, HSOR and HQSOR groups

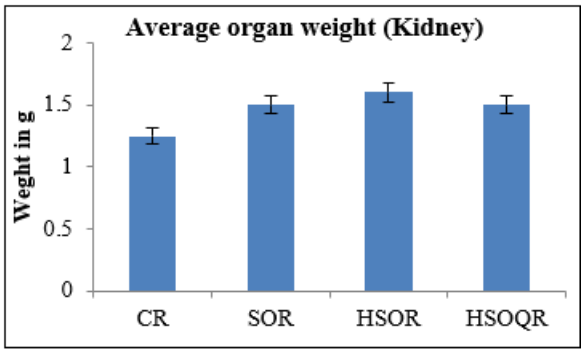

Figure 6: Average weight of kidney of CR, SOR, HSOR and HQSOR groups

The SOR, HSOR and HSOQR groups showed slightly increase in the heart weight as when compared with the CR group. Further HSOR shows slightly higher increase in the heart weight compared to SOR and HSOQR as in Fig.4. The liver weight gain for the HSOR group was least compared to the control group and other group as shown in the Fig.5. Compared to the control group the oil fed groups showed increase in kidney weight generally and slightly higher gain weight is recorded for HSOR groups as shown in Fig.6. The interesting changes in the organ weight (liver, kidney and heart) for HSOQR group may be due the histopathological changes explained in the later stages.

Blood pressure

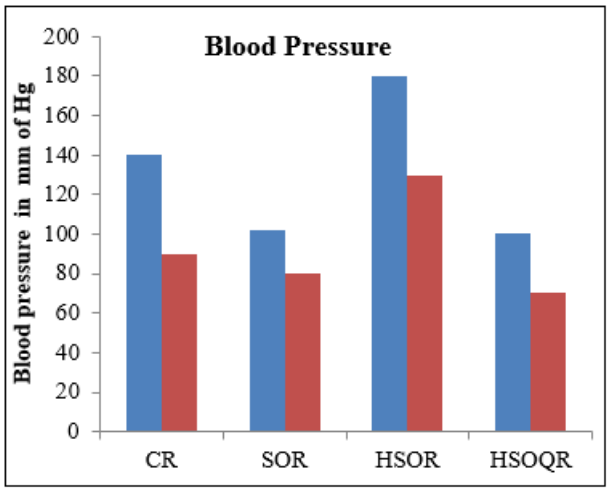

Figure 7: Average blood pressure CR, SOR, HSOR and HQSOR, Sp (systolic blood pressure): blue, Dp (diastolic blood pressure): red

In the present study it is observed that CR, SOR and HSOQR had a blood pressure almost in the normal range (60 - 90) systolic (Sp) / (75 - 120) diastolic (Dp). HSOR group showed an increase in blood pressure (130/180) both Dp and Sp as observed in Fig.7. A previous study has also reported that consumption of repeatedly heated palm oil resulted in high blood pressure with necrosis of cardiac tissue in experimental rats (50). This may be due to over - production of oxidative compounds in the fried oil by the process of repeated deep frying. Frequent heating of cooking oil is capable of making it more vulnerable to lipid peroxidation, thus producing extreme level of invasive by - products (51). In addition, deep frying also reduces the antioxidative vitamin E constituents in palm oil (52). In this case also it may expect that the natural antioxidants like sesamol, sesamin and sesamolin might have demolished during frying process and in the presence of synthetic antioxidant (TBHQ), the SO is more stable and with less oxidized products. Hence, HSOQR groups have shown blood pressure similar to SOR and CR groups. Therefore, recycled deep - frying oil may reduces the antioxidant capacity and elevate oxidative stress in rats (53)

Table 5: Comparison of platelet indices (PLT, P - LCR, MPV and PDW) in the CR and SOR, HSOR and HSOQR groups

Parameter	CR	SOR	HSOR	HSOQR
PLT (10^3/mm^3)	789.5± 36.21	710.5±16.5	949.5±20.36	895±18.53
P - LCR (%)	8.95±1.2	9.8±0.8	12.7±0.9	9.3±1.1
MPV (fL)	7.20±0.11	7.0±0.07	7.2±0.08	7.3±0.11
PDW (fL)	8.7±0.4	8.6±0.2	9.1±0.3	8.8±0.21

Values are mean of 6 animals ± SEM, p< 0.05 vs Control, n = 6.
PLT: Platelet Count; P - LCR**:** Platelet larger cell ratio; MPV: Mean platelet volume; PDW: Platelet distribution width

The platelet indices PLT, P - LCR, MPV and PDW observed in the CR, SOR, HSOR and HSOQR groups is represented in Table 5. Slight variations, platelet larger cell ratio, mean platelet volume and platelet distribution width in HSOR group except platelet counts was observed. Abnormalities in platelet number are an indication of a defect in primary hemostasis. Platelet count (PC) was significantly increases in heated oil fed groups, but least in fresh oil fed groups. An increase in platelet number above normal, serves as a marker of vascular disease (54) (Vidwan 2010). Mohammad Anwar et al., 2010 reported significant increase in platelet count in high fat diet fed rabbits in comparison to control rabbits. In our study, the platelet count in rats fed heated SO is more compared to the other groups. An increased in P - LCR percentage is seen in conditions where there is platelet destruction. Mean platelet volume (MPV) is an indicator of platelet function and increased MPV is one of the risk factors for myocardial infarction, cerebral ischemia and transient ischemic attacks (55). However, there are no significant changes in P - LCR and mean platelet volume (MPV) for all groups of rats investigated. Also there is insignificant change in platelet distribution width (PDW) for all of rat groups.

Table 6: Comparison of PCV, WBC, RBC, Hb, HCT, Lymphocyte % and Lymphocyte count for CR, SOR, HSOR and HSOQR groups

Parameter	Control	SOR	HSOR	HSOQR
WBC (x10^3/µl)	8.7 ±0.92	5.9±0.62	10.75±0.54	5.1±0.61
RBC (x10^6/µl)	9.19±0.94	10.79±0.65	9.09±0.82	10.05±0.78
Hb (g/dl)	16.4± 0.64	17.5±0.52	15.3±0.61	15.4±0.54
HCT (%)	54.3± 0.64	57.5± 0.55	50.6± 0.48	57.5± 0.56
Lymphocyte (%)	84.8±2.1	82.5±2.11	79.45±1.5	78.1± 1.69
Lymphocytes count (x10^3/µl)	7.35±0.41	6.1±0.35	11.9±0.32	6.9±0.31

Values are mean of 6 animals ± SEM, p < 0.05 vs Control, n = 6.
WBC: White blood cells; RBC: Red blood cells; HGB: Hemoglobin; (HCT): Hematocrit percent or packed cell volume.

The measured PCV, WBC, RBC, Hb, HCT, Lymphocyte % and Lymphocyte count for CR, SOR, HSOR and HSOQR groups is tabulated in Table 6. The WBCs count is found to be higher in HSOR group compared with the CR, SOR and

HSOQR groups. Also it is found that the WBC count is almost same in SOR and HSOQR groups. The role of WBCs is protecting the body against the different infections. The increase was probably due to increase in the lymphocyte count, as noticed in Table 6. The lymphocytes represent the first defense line against the different infections in the body. In the rats, the lymphocyte is the prevailing white cells, therefore its increase is expected to increase the total WBC. The increase of WBC may be also due to eosiniphilic and neutrophilic cells, which have been found as infiltrate in kidney tissues as shown in figure 14 (B) and (C). Neutrophils are the major granulocytes to be activated when the body is invaded by bacteria and they provide the first line of defense against invading microorganisms (56) Higher level in WBC causes inflammation. If an infection develops, WBCs attack and destroy the bacteria, virus, or other organism causing it. The number of WBCs sometimes used to observe how the body is dealing with cancer treatment. Heated sesame oil may cause infection due to the formation of FFA, and polar compound formation which is more in HSO as observed in the Table 1.

RBCs count shows an insignificant decrease when rats were fed with heated SO compared with the control. Haemoglobin also did not alter significantly of all the groups of rats.

Table 7: Comparison of red blood cell absolute values MCV, MCH, MCHC and RDW in CR, SOR, HSOR and HSOQR groups

Parameter	Control	SOR	HSOR	HSOQR
MCV (f/l)	56.5± 1.91	56.5±1.81	57.5±1.52	57.35±1.23
MCH (pg)	17.05±0.44	17.55±0.45	16.45±0.41	15.45±0.42
MCHC (g/dl)	30.2±0.51	30.45±0.31	29.6±0.45	26.9±0.24
RDW (f/l)	28.8±0.81	28.05±0.56	29.45±0.52	28.9±0.21

Values are mean of 6 animals ± SEM, a = p< 0.01, b = p< 0.025, c = p< 0.05 vs Control, n = 6.
MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: MCH concentration; RDW: Red cell distribution width

The measured MCV, MCH, MCHC and RDW values in CR, SOR, HSOR and HSOQR groups are tabulated in Table 7. There **is** slight increase in MCV is found in HSOR and HSOQR groups. The MCV shows the size of RBCs and increase in size of RBCs indicates changes in morphology and deformability. It also confirmed by a slight increase in the red cell distribution width (RDW). One of the index for diagnosing anemia is mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Both MCH and MCHC showed a slight decrease in value in both the HSOR and HSOQR groups. Low MCHC is an indicator of hypochromia in early iron deficiency and also MCH falls as the hypochromia develops (57). However, there are insignificant variations found in MCV, MCH, MCHC and RDW values for all the groups of rats.

4. Conclusions

Peroxide value, free fatty acid value, total polar compounds and totox values was found to be increase for repeatedly due to thermal oxidation which indicates unsafe for consumer health. These changes in physico chemical parameters of heated oils confirm the oxidation of edible oil and formation of free radicals. The animals fed with HSO shows significant changes in the organ weight while that of HSOQ show insignificant changes. Haematological data such as RBC, WBC, HGB, HCT, MCV, MCH, MCH, MCHC, PLT, LYM, RDW, PDW, MPV and P - LCR indicated the toxicity level of HSO. The histopathological study of organs like liver, heart, and kidney indicated the level of toxicity is more in HSOR group as compared to SOR and HSOQR groups. From the present study, it can be concluded that fresh SO gives benefits; HSO can be toxic and can cause considerable damage to the vital organs of the experimental animals whereas SO with 200 ppm of TBHQ is more safe and less toxic comparatively.

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