

Thermally oxidized corn oil adversely affects serum biochemistry, blood hematology and liver histopathology of rabbits

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Abstract:

Corn oil was thermally oxidized at 130 ± 10 °C for consecutive five and 10 hours. Rabbits were used as experimental animals. Rabbits were divided into eight groups including control. Normal/non-oxidized corn oil and thermally oxidized corn oil was given to rabbits in different doses. Oxidized corn oil lipid profile showed increase level of total cholesterol, triglycerol and LDL. However no significant increase was observed in HDL concentration. Increase was observed in ALT concentration while decrease was observed in glucose concentration in oxidized corn oil fed rabbits. Decrease level of TRBs, hemoglobin, HCT,

while increase in WBCs count was observed in oxidized corn oil fed rabbits. Liver histopathology showed mild dilation, necrosis changes and proliferation of bile duct in oxidized corn oil fed rabbits. Normal corn oil fed rabbits showed no significant differences comparing control. The present study concluded that use of oxidized corn oil and reuse of oxidized corn oil should be avoided for long time.

Key words: Oxidized lipids, Oxidized Corn Oil, Hematology, Histopathology, Blood Biochemistry, Rabbits.

Introduction

Maize is one of the most important plant which provides food, fodder and feed. It also provides raw material for alcoholic beverages, protein, biofuel, cosmetics and for other industrial products. It also provides edible oil (Singh and Langade, 2013). Milling industries of maize provides protein, starch and other important minerals and vitamins (Burge and Duensing, 1989, Johnston et al., 2005). Corn oil is mostly obtain from the wet milling of maize by products, or it may be obtain from the corn germ (embryo), which is rich portion of oil (Moreau and Hicks, 2005). Corn oil is very important for body which provides essential fatty acid like linoleic acid and linolenic acid which is necessary for growth, immune system, skin and cell membrane. It also acts as transporter for fat soluble vitamins (Fasina et al., 2006). The oxidative stability of oil is resistance during storage and processing. Less saturated oil oxidize more than saturated oil (Lenhard and Parker, 1987). With the increase of unsaturation there is increase in primary oxidation products (Martin-Polvillo et al., 2004). Naturally this oil composed of naturally antioxidant, triacylglycerol (TAG) and small amount of cholesterol. With the frying of food TAG and cholesterol oxidized to hydroperoxides, epoxides and hydroxide

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(Kamal-Eldin, 2006). Oxidation have a significant effect on the properties and composition of fats or oils (Zeb and Mehmood, 2012). The oxidative products produced during oxidation cause hepatic damage and increasing the growth of hepato carcinoma (Rohr-Udilova et al., 2008).

Corn oil is used as cooking oil in Pakistan. It is used in frying different kinds of food products. Reuse of corn oil is often carried out in certain area, which increase the oxidation time of the oil. Increase in oxidation time enhance epoxide and peroxide which are harmful to human body. The purpose of this study is to fine the possible toxic effect of oxidized corn oil on experimental animals.

Method and Material

Corn oil was purchased from local market Chakdara Dir Lower Pakistan. It was thermally oxidized on hot plate at 130 ± 10 °C for consecutive five and ten hours. After oxidation it was stored at -21 °C to stop further oxidation. The study plan was approved from ethical board and graduate studies committee (GSC) Department of Biotechnology University of Malakand.

Animal Feeding

Rabbits were used as experimental animal. Male rabbits weighing 1.6 ± 0.2 kg were bought from local market and acclimatized at the bio-park of University of Malakand. The oils were given orally. The rabbits were divided into the following groups.

- Group A: Fed with 1 g/kg 5 hours oxidized corn oil.
- Group B: Fed with 2 g/kg 5 hours oxidized corn oil.
- Group C: Fed with 3 g/kg 5 hours oxidized corn oil.
- Group D: Fed with 1 g/kg 10 hours oxidized corn oil.
- Group E: Fed with 2 g/kg 10 hours oxidized corn oil.
- Group F: Fed with 3 g/kg 10 hours oxidized corn oil.
- Group G: Fed with 1.5 g/ kg normal corn oil.

Group H: No oil (Control)

Biochemical analysis

After 15 days the rabbits were weighted again, blood samples were obtain from venipuncture of the jugular vein. The blood were stored in SSGT tubes for serum analysis and in EDTA containing tube for hematological parameter. Same procedure was repeated after 30 days. Biochemical analysis was carried out by using UVvis-1700 spectrophotometer (Shemadzu, Japan) using HUMAN (Germany) kits via six point standard calibration curve with standard protocol. LDL-c concentration were calculated according to standard equation (Friedewald et al., 1972).

$$\text{LDL-cholesterol} = \text{Cholesterol} - \text{HDL- TG/5}$$

Hematology and Histopathology

Hematology or blood profile was done by automatic digital machine (Sysmex Kx-21). After sacrificing the rabbits, different organs i.e. liver, kidneys and heart were weighted and stored in 10 % formalin solution. Liver tissues were stained, sectioned and processed according to standard protocol (Luna, 1968). The slides have been studied by electric microscope model No. M 7000 D; SWIFT, Japan and pictures have been captured by digital camera for microscope DCM 130 (USB 2.0), resolution 1.3 MP commonly known as CCT camera.

Peroxide Value and Radical Scavenging assays

Peroxide value (POV) of the corn oil were found according to AOCS standard protocol (AOCS, 1998). Radical scavenging assay (RSA) were measured according to standard protocol with small modification (Lee et al., 2007). A 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in ethyl acetate of 0.1 mM, was freshly prepared. Mixing five ml of 0.1 mM DPPH solution with 56 μ L of corn oil and incubated for 30 min in dark. After incubation the absorbance were measured with UVvis-1700

spectrophotometer at 515 nm. The absorbance of the blank solution were also measured. The % RSA were calculated according to following equation.

$$\% \text{ RSA} = ((\text{As}-\text{Ac})/\text{Ac}) \times 100$$

Where Ac is the absorbance of the control and As is the absorbance of the test sample.

Statistical Analysis

Data were analyzed by one way analysis of variance (ANOVA) using “Tukey: Compare all pairs of column” method of $P < 0.05$ using GraphPad Prism 5 for windows version 5.03 (GraphPad Software, Inc, Dec 10, 2009).

Results and Discussion

POV and RSA

Measurement of hydroperoxides formed during oxidation of fats and oil is called peroxide value. The hydroperoxides may further oxidized to secondary oxidation products which having varying chain length (Zeb and Mehmood, 2012). Fig 1 shows that the peroxide value of corn oil increase with increase in oxidation time. There was a small increase (0-25 meq/kg) with in first five hour of oxidation and after ten hours the increase was very large (25-190 meq/kg). At this stage the antioxidant containing in corn oil have completely oxidized which protect the oil from oxidation, and further increase in oxidation time may increase the production of hydroperoxides (Zeb and Murkovic, 2011). RSA is a parameter used to measure the potential of antioxidant in oil or fats (Zeb and Mehmood, 2012). Fig 2 shows that the radical scavenging activity of non-oxidized corn oil (48 %) were decreases after five hour of oxidation (32 %). As the oxidation time increased to ten hours, greater decrease (13 %) was observed. Thermal oxidation destroy the antioxidant activity of oil and fats (Achir et al., 2010, Karabulut, 2010). A significant decrease ($P < 0.05$) in RSA after

five hours of oxidation. This may be due to the oxidation of all antioxidant in corn oil which may protect the oil from oxidation (Zeb and Mehmood, 2012).

Change in Body Weight

Studying different parameter, growth is one of them which have been affected by a specific diet. The present results showed when oxidized corn oil was given to the rabbits, loss of weight were observed as shown in Fig 3. However weight loss was directly proportional to oxidation time and dose of the oxidized corn oil. No significant effect was found when non-oxidized corn oil was given. Diets containing safflower oil cause an increase in thermogenesis (Ide and Sugano, 1988) and uncoupling protein content of brown adipose tissue and results in lowering of body weight (Maki et al., 2002). In rats, high-fat feeding leads to an increase in basal and hormone-stimulated lipolytic activity which leads to body weight loss (Berger and Barnard, 1999). The results were in agreement with Vecera et al (2002), who stated that the effect of PUFA has been related to anitadiposity (Večeřa et al., 2003). Similarly Jeffery et al (1996) also showed that PUFA results lower body weight. Similar results were observed in weight loss while feeding diet rich in essential fatty acid, sunflower oil and high fat diet that leads to lipolysis (Jeffery et al., 1996).

Effect on Serum Lipids Profile

Three types of cholesterol found in blood i.e. HDL, LDL and VLDL. Along with cholesterol the body contains neutral fat which is called triglyceride (TG). Biochemical analysis showed that when oxidized oil was given to the rabbits increase in serum total cholesterol, TG and LDL were observed as compared to control. A significant increase in serum cholesterol was found as shown in Table 1. Non-significant increase were observed when non-oxidized corn oil were given alone. Jaarin et al (2006) studied that a significant increase in serum total

cholesterol were found in rats when heated vegetable oil were given (Jaarin et al., 2006). The results also correlate the study of Shastry (Shastry et al., 2011), they found that the serum total cholesterol level increased when the rats were fed with fresh palm oil and reused palm oil compared to control group. Earlier study suggested that the effect of heated oil at 210 °C have increasing effect on serum total cholesterol (Chacko and Rajamohan, 2011).

The present study showed increase in serum TG concentration. The increase was significant ($P < 0.05$) dependent on dose and oxidation time of the corn oil as shown in Table 1. The present study was in agreement with the results of Shastry (Shastry et al., 2011) and Chako (Chacko and Rajamohan, 2011). Hussain studied that administration of *Eruca sativa* seed oil have TG decreasing effect on alcoholic injured liver rats, which was inverse to our observation (Hussein et al., 2010). Al-Attar studied increase in serum TG lever in corn oil fed rats (Al-Attar, 2014).

The LDL concentration was also found to be increasing significantly at $P < 0.05$ and dose dependent. While the concentration of HDL have no significant difference comparing control as shown in Table 2. Diet rich in fats increase the LDL concentration accordance to previous finding (Tholstrup et al., 2011, Karpe, 1997). The increase in hydroperoxides in body have increasing effect on LDL concentration (Zeb and ur Rahman, 2012). Kumar et al stated that 5 % ghee in diet may not significantly affected the HDL-cholesterol concentration in rats (Kumar et al., 1999). It was also found that rabbits fed with oxidized ghee have no significant effect on HDL-c concentration (Zeb and Mehmood, 2012). Earlier study showed that after supplementation of PUFA-rich diet decrease in HDL-c concentration was found (Nordøy and Goodnight, 1990).

Serum Glucose

Glucose is the main and rapid precursor of energy in animal body. Glucose is the circulating carbohydrate of blood stream and energy yielding nutrient (Cox and Nelson, 2004). The present study showed a decrease in serum glucose level. The decrease were not significant in all groups. A significant difference ($P < 0.05$) were observe between Control (H) and F group, and G and F group as shown in Table 1. Decrease in serum glucose was found in rabbits fed with oxidized ghee. The increase level of LDL, total cholesterol and TG may decrease the concentration of serum glucose level (Zeb and Mehmood, 2012). Previous studies have suggested a decrease in serum glucose level of oxidized tallow fed rabbits. Chances of hypoglycemic condition increases with oxidation and hence weight loss occurs (Zeb and ur Rahman, 2012).

Serum ALT

Serum alanine aminotransferase is also called serum glutamic pyruvic transaminase (SGPT). It is found in many tissue like skeletal muscles, heart and liver. The transfer of amino group from α -ketoglutarate to form glutamate and pyruvate is catalyzed by ALT. Concentration of ALT in serum is important for evaluation of liver and heart damage cause by infection or drug. The present study showed an increase in ALT concentration. The increase was not significant at all but significant ($P < 0.05$) when 3 g of oxidized corn oil was given, comparing control as shown in Table 2. Previous studies showed that increase in SGPT were found in rats fed with oxidized ghee (Rahman et al., 2012).

Hematology

Blood is a circulatory fluid of the vascular system. Blood is composed of plasma and blood cells. The plasma contain electrolytes and other protein which maintain the flow of blood. The blood cells composed of red blood cells (RBCs), white blood

cells (WBCs) and platelets. RBCs is responsible for transport of oxygen to all parts of the body while WBCs fights again foreign invaders. WBCs further composed of neutrophil, basophil, eosinophil, lymphocytes and monocytes. Platelets is responsible to stop bleeding during injuries. Hematological concentration showed that when oxidized corn oil were given to the rabbits, a significant decrease were observe in TRBc, hemoglobin, HCT and platelets counts as shown in Table 3. The decrease was directly proportional to the dose and oxidation time of the oxidized corn oil. However non-significant difference was observe when non-oxidized oil were given alone as shown in Table 3. Earlier study showed a decrease in RBCs, Hb and Platelets were observed when oxidized *Eruca sativa* seed oil were given to the rabbits (El-Nattat and El-Kady, 2007). Significant increase in WBCs count were observed in oxidized corn oil fed rabbits as shown in Table 3. The study correlates with the previous study which stated that increase in WBCs count were found in thermally oxidized fed rats. Oxidized palm oil may cause liver damage which increase WBCs level (Osime et al., 1994, Finlayson et al., 1999).

Histopathology

Liver histopathology of group “A” having 1 g OCO5/kg showed hepatic tissue with almost intact architecture; however focal area portal tract showed proliferation of bile duct. The hepatocytes were normal in appearance. Sinusoids showed mild degree of dilation at places of hepatic tissue and edematous change of bile duct occurred as shown in Fig 3(A). Group “B” having 2 g OCO5/kg liver histopathology showed hepatic tissue revealing necrotic changes and increased lymphocytic infiltrate. However portal tract showed lymphatic infiltration. Sinusoids showed mild degree of dilation at places of hepatic tissue and edimatous change of bile duct occurred as shown in Fig 3(B). The liver histopathology of group “C” having 3 g OCO5/kg showed hepatic tissue revealing coagulative necrosis changes.

The bile ducts were dilated and some were filled with crystalloids, while some of ducts showed bile duct dilation as shown in Fig 3(C). Group “D” having 1 g OCO10/kg, liver histopathology showed hepatic tissue revealing bile duct dilation. In some places moderate cholestasis also been found in hepatic tissue as shown in Fig 3(D). Liver histopathology of group “E” having 2 g OCO10/kg showed hepatic tissue revealing coagulative necrosis changes. The bile ducts were dilated and some were filled with crystalloids, while some of ducts showed proliferation. However portal tract showed lymphatic infiltration as shown in Fig 3E. Group “F” having 3 g OCO10/kg, liver histopathology showed edematous change of bile duct along with mild dilation at hepatic tissue as shown in Fig 3 (F). The liver histopathology of group “G” having 1.4 g NOCO/kg body weight and group “H” taken as control, showed that the architecture appears to be almost normal, revealing central vein, hepatic cords and portal tracts. The overall architecture appears to be normal as shown in Fig 3 (Control). A few changes like necrotic area may be due to mild autolysis was observed in group G as shown in Fig 3(G). Widened sinusoids and sever necrosis in hepatocytes of liver were observe when rats were fed with oxidized oil (Jimoh and Odutuga, 2004). Congestion in rats liver were observed in oxidized palm oil fed rats (Osim et al., 1994). Earlier studies suggested that the reuse of sunflower oil may cause chronic inflammatory cell infiltration and swollen liver cells in Wistar rats (Shastry et al., 2011).

Conclusion

The present results concluded that when corn oil was oxidized increase in POV and decrease in RSA occurs. These changes in properties shows that upon oxidation antioxidant activity decreases, and further more usage of oxidized cord oil is harmful to health. Normal corn oil have no significant effect on

rabbits, comparing control, however use of oxidized and reuse of corn oil for long period of time should be avoided.

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Islam Uddin, Sultan Ahmad, Iftikhar Ul Haq, Said Nawab, Meftah Uddin- **Thermally oxidized corn oil adversely affects serum biochemistry, blood hematology and liver histopathology of rabbits**

Table 1. Effect of oxidized corn oil on serum glucose, serum cholesterol and serum TG concentration of rabbits

Dose/kg	Body Wt /Group	Serum Glucose Conc. (mg/dl)		Serum Cholesterol Conc. (mg/dl)		Serum TG Conc. (mg/dl)	
		Mean ± SD		Mean ± SD		Mean ± SD	
		15 Days	30 Days	15 Days	30 Days	15 Days	30 Days
1 g OCO5/kg (A)		77.7 ± 1.5	75.3 ± 3.8	74.7 ± 2.5	75.3 ± 2.5	131.3 ± 4.9	134.0 ± 7.5
2 g OCO5/kg (B)		71.0 ± 3.0 ^a	69.7 ± 4.7	79.7 ± 3.1	81.0 ± 3.6	134.7 ± 3.5 ^b	139.3 ± 4.7
3 g OCO5/kg (C)		68.7 ± 4.7 ^a	62.0 ± 3.6 ^{ab}	85.3 ± 4.0 ^{ab}	90.3 ± 5.7 ^{abc}	134.7 ± 3.2 ^{ab}	139.3 ± 3.8 ^{bc}
1 g OCO10/kg (D)		65.0 ± 5.3 ^{abc}	60.7 ± 2.5 ^{bc}	84.0 ± 4.6 ^{ab}	92.0 ± 3.0 ^{abc}	139.0 ± 6.0 ^{ab}	152.3 ± 3.8 ^{abc}
2 g OCO10/kg (E)		60.3 ± 4.2 ^{abc}	59.3 ± 6.0 ^{abc}	82.3 ± 3.5	95.0 ± 5.3 ^{abc}	147.3 ± 4.7 ^{abc}	157.7 ± 5.9 ^{abc}
3 g OCO10/kg (F)		59.3 ± 7.1 ^{abc}	54.7 ± 5.9 ^{abc}	90.7 ± 7.8 ^{abc}	103.7 ± 5.7 ^{abc}	146.3 ± 4.5 ^{abc}	170.7 ± 6.0 ^{abc}
1.5 g NCO/kg (G)		79.3 ± 4.0	82.0 ± 3.6	70.7 ± 2.5	72.0 ± 3.0	121.7 ± 2.5	125.7 ± 6.1
Control (H)		89.3 ± 3.2	85.3 ± 5.7	70.7 ± 2.5	70.7 ± 3.2	119.0 ± 3.0	122.7 ± 7.4

^aSignificantly different from Control, ^b significantly different from G, ^c significantly different from A.

Abbreviation OCO5=oxidized corn oil for five hours, OCO10=oxidized corn oil for 10 hours & NCO=non-oxidized corn oil.

Table 2. Effect of oxidized corn oil on serum HDL-c, serum LDL-c and ALT concentration of rabbits

Dose/kg	Body Wt /Group	Serum HDL-c Conc. (mg/dl)		Serum LDL-c Conc. (mg/dl)		ALT	
		Mean ± SD		Mean ± SD		Mean ± SD	
		15 Days	30 Days	15 Days	30 Days	15 Days	30 Days
1 g OCO5/kg (A)		31.0 ± 2.0	31.0 ± 1.7	17.4 ± 0.9	17.5 ± 3.5	76.3 ± 1.5	76.3 ± 3.1
2 g OCO5/kg (B)		31.3 ± 1.5	31.7 ± 2.5	21.4 ± 1.4	21.5 ± 5.3	75.7 ± 4.2	82.3 ± 1.5
3 g OCO5/kg (C)		31.7 ± 3.5	30.7 ± 1.5	26.7 ± 7.1	31.8 ± 5.9 ^{ab}	77.7 ± 3.5	79.3 ± 2.1
1 g OCO10/kg (D)		32.0 ± 4.0	32.7 ± 3.5	24.2 ± 8.0	28.9 ± 0.8 ^a	77.3 ± 2.1	80.3 ± 4.2
2 g OCO10/kg (E)		33.0 ± 4.0	32.3 ± 3.8	19.9 ± 6.5	31.1 ± 8.6 ^{ab}	79.7 ± 3.5	84.7 ± 2.5 ^a
3 g OCO10/kg (F)		31.0 ± 3.6	32.7 ± 3.2	30.4 ± 4.4 ^{ab}	36.9 ± 5.1 ^{abc}	79.0 ± 6.0	89.7 ± 5.9 ^{abc}
1.5 g NCO/kg (G)		30.7 ± 2.1	31.7 ± 2.5	15.7 ± 4.2	15.2 ± 4.0	73.0 ± 4.0	74.7 ± 2.5
Control (H)		32.7 ± 3.5	33.3 ± 2.1	14.2 ± 3.2	12.8 ± 3.7	73.3 ± 4.7	73.3 ± 4.5

^aSignificantly different from Control, ^b significantly different from G, ^c significantly different from A.

Abbreviation OCO5=oxidized corn oil for five hours, OCO10=oxidized corn oil for 10 hours & NCO=non-oxidized corn oil.

Table 3. Effect of oxidized corn oil on hematological concentration in rabbits.

Dose/kg Body Wt /Group	TRBCs Conc. (× 10 ⁶ /µl)		TLC Conc. (× 10 ³ /µl)		Blood Hemoglobin Conc. (mg/dl)		HCT (%)		Platelets Conc. (× 10 ³ /µl)	
	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
	15 Days	30 Days	15 Days	30 Days	15 Days	30 Days	15 Days	30 Days	15 Days	30 Days
1 g OCO5/kg (A)	4.3 ± 0.2	4.0 ± 0.5	4.6 ± 0.3	4.7 ± 0.4	11.7 ± 0.2	11.6 ± 0.3	42.3 ± 1.5	40.7 ± 0.6	311.3 ± 8.5	297.0 ± 7.0
2 g OCO5/kg (B)	3.7 ± 0.1	3.4 ± 0.2	4.9 ± 0.2	5.0 ± 0.1	11.2 ± 0.4	11.0 ± 0.3	39.3 ± 2.1	38.3 ± 1.2	304.3 ± 5.5	300.0 ± 9.5
3 g OCO5/kg (C)	3.4 ± 0.3 ^{abc}	3.3 ± 0.2 ^{ab}	4.9 ± 0.1 ^{ab}	5.1 ± 0.2 ^{ab}	11.7 ± 0.3 ^{ab}	11.1 ± 0.4	39.0 ± 2.0	36.3 ± 3.1 ^{ab}	302.0 ± 14.4	292.7 ± 12.7 ^{ab}
1 g OCO10/kg (D)	4.1 ± 0.2 ^{ab}	3.6 ± 0.3 ^{ab}	5.0 ± 0.2 ^{ab}	5.2 ± 0.3 ^{ab}	10.9 ± 0.4 ^{ab}	10.5 ± 0.2 ^{ab}	34.7 ± 3.8 ^{abc}	33.3 ± 2.5 ^{abc}	292.7 ± 4.2 ^{ab}	279.0 ± 8.0 ^{ab}
2 g OCO10/kg (E)	3.6 ± 0.2 ^{abc}	3.3 ± 0.3 ^{ab}	5.0 ± 0.3 ^{ab}	5.2 ± 0.3 ^{ab}	9.8 ± 0.7 ^{ab}	09.4 ± 0.5 ^{abc}	35.7 ± 2.5 ^{abc}	32.0 ± 1.7 ^{abc}	290.0 ± 11.5 ^{ab}	279.3 ± 8.5 ^{ab}
3 g OCO10/kg (F)	3.1 ± 0.1 ^{abc}	3.0 ± 0.1 ^{abc}	5.2 ± 0.3 ^{ab}	5.2 ± 0.1 ^{ab}	9.5 ± 0.2 ^{ab}	09.2 ± 0.7 ^{abc}	33.0 ± 2.6 ^{abc}	29.3 ± 1.5 ^{abc}	285.0 ± 7.0 ^{abc}	268.0 ± 12.5 ^{abc}
1.5 g NCO/kg (G)	4.9 ± 0.2	4.8 ± 0.2	3.9 ± 0.1	3.9 ± 0.3	12.1 ± 0.3	12.0 ± 0.1	43.7 ± 1.5	44.7 ± 2.5	323.7 ± 4.7	320.3 ± 9.1
Control (H)	5.4 ± 0.3	5.1 ± 0.2	3.8 ± 0.1	3.7 ± 0.1	12.1 ± 0.2	12.3 ± 0.2	43.7 ± 1.5	45.0 ± 2.6	323.7 ± 3.8	321.0 ± 4.4

^aSignificantly different from Control, ^b significantly different from G, ^c significantly different from A.

Abbreviation OCO5=oxidized corn oil for five hours, OCO10=oxidized corn oil for 10 hours & NCO=non-oxidized corn oil.

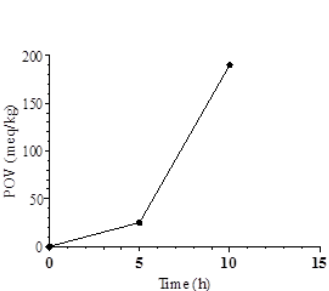


Fig 1. Change in peroxide value (meq/kg) of thermally oxidized corn oil.

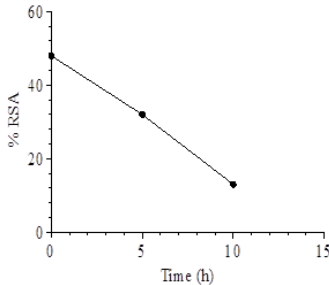


Fig 2. Change in radical scavenging assay (RSA) of corn oil with oxidation.

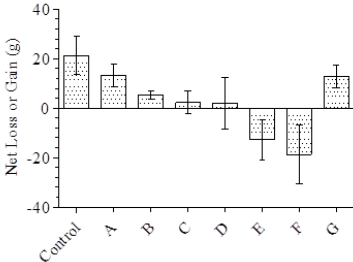


Figure 3. Change in body weight of rabbits (g).

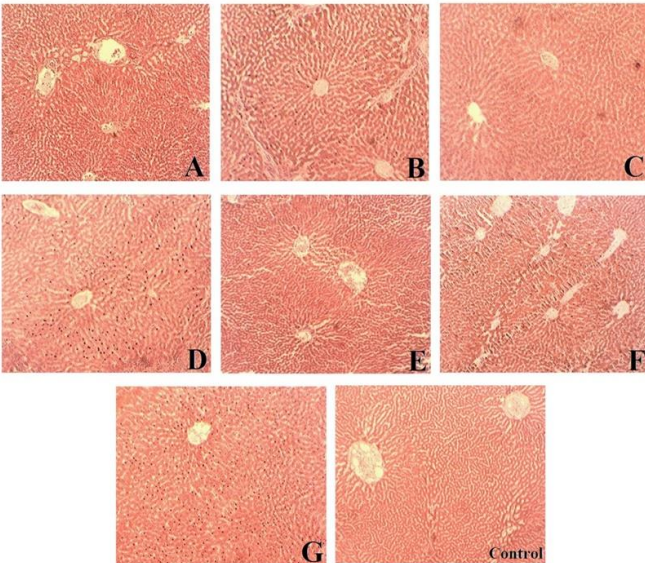


Fig 4. Liver micrograph of all groups. (Magnification 100X)