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The Effects of *Camellia sinensis* (Green Tea) Against Oxidized *Helianthus annuus* (Sunflower) Oil in Rabbits

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The lipids oxidation is essential, keeping in view the taste, quality of nutritional components, and toxicity of lipids. We used oxidized sunflower oil combined with green tea to check its effects on hematological and serum biochemical parameters and liver in rabbits. Sunflower oil was oxidized continuously for 5 hours on hot plates at 100^0C. The rabbit groups were made, one group was fed a regular diet, and the other was fed with oxidized oil (2ml/kg body weight). Three groups were provided with green tea (50-50 ratio) at the dose rate of 100, 200, and 300 mg/kg combined with oxidized sunflower oil (2m/kg). Green tea alone was fed to two groups (200 mg and 100 mg/kg). The feeding regimen was continued for twenty-one days. The blood samples were collected on days zero, 11th, and 21st to analyze hematological and biochemical parameters. At the end of the experiment, one rabbit from each group was slaughtered, and the liver was extracted for histopathology. In the groups fed with oxidized sunflower oil alone or combined with green tea at various doses, significant alterations ($P<0.05$) in hematology and biochemical indices were observed. The high doses of green tea improved the alteration. No ill effect was observed in the groups fed green tea alone. Oxidized sunflower oil alone led to cholestasis and fatty changes in the liver, while green tea minimized the toxic effects confirming its anti-oxidant properties**.**

Keywords: Oxidized sunflower oil, green tea, anti-oxidants, rabbits.

INTRODUCTION

Various medicinal plants are used to treat different disease conditions (Gupta et al. 2011). Utilizing plants in part or entirely has low cost and fewer side effects (Patel et al. 2013). Sunflower is one of the most broadly developed oil crops on the planet. In 1998, the seed world creation was around 28.5 million tons and, as consumable vegetable oil (Flagella et al. 2002). Sunflowers (*Helianthus annuus* L.) are utilized in the eating regimen, for oil, to acquire colors, for clinical purposes, and as a fancy plant (Jocic et al. 2015).

Oil oxidation also obliterates essential unsaturated fats and creates harmful mixtures and oxidized polymers. Oxidation of oil is vital for the attractiveness, wholesome quality, and poisonousness of edible oils. Different substance systems, autoxidation, and photosensitized oxidation are answerable for the oxidation of edible fats during handling and capacity relying on oxygen (Choe and Min 2006).

The synthetic structure of Green tea is mind-boggling: proteins (15-20% dry weight) whose chemicals comprise a significant division; amino acids (1-4% dry weight, for example, theanine or 5-Nethylglutamine, glutamic corrosive, tryptophan, glycine, serine, aspartic corrosive, tyrosine, valine, leucine, threonine, arginine, lysine; sugars (5-7% dry weight) like cellulose, gelatin, glucose, fructose, sucrose; lipids as linoleic and - linolenic acids; sterols as shame sterol; nutrients (B, C, E); xanthic bases like caffeine and theophylline; colors as chlorophyll and carotenoids; unpredictable mixtures as aldehydes, alcohols, esters, lactones, hydrocarbons, and so forth; minerals and minor components (5% dry weight) like Ca, Mg, Cr, Mn, Fe, Cu, Zn, Mo, Se, Na, P, Si, Ni, K, F, and Al. Because of the incredible significance of the mineral presence in tea (Cabrera et al. 2006). Green tea contains numerous polyphenols known as catechins, including epigallocatechin-3 gallate (EGCG), epigallocatechin (EGC), and epicatechin-3 gallate (ECG) [7] that have organic action in cell reinforcement against angiogenesis, and antiproliferative examines possibly applicable to the anticipation and therapy of different types of malignant growth (Cooper et al. 2005).

In the current study, we studied the effects of oxidized sunflower oil on liver and kidney function and blood parameters. We also plaid the synergistic effects of green

tea administered in combination with oxidized sunflower oil.

MATERIALS AND METHODS

Sunflower oil was purchased from the Matta, Swat, Khyber Pakhtunkhwa, Pakistan. The sunflower oil was selected based on its high linoleic acid and oleic acid contents. Rabbits *(Oryctolagus cuniculus)* were used as experimental animals, and sixty (60) rabbits were purchased from the local market. The rabbits were transferred to the Bio-Park of the Biotechnology Department, the University of Malakand, for acclimatization. The Ethical Board approved the study of the Biotechnology Department. The standard grade of EDTA tubes, gel tubes, syringes, surgical kit, Formaldehyde, and Chloroform was used.

Thermal Oxidation of Oil and Feeding Regime

For regular 5 hours, sunflower oil samples were kept on hot plates at 100 ^{0}C, and after oxidation, the samples were transferred to the refrigerator and were kept at -20 ^{0}C to avoid deterioration. Rabbits were divided into nine groups based on body weight, and each group had three rabbits.

Group A: Fed with regular diet, Group B: Fed with oxidized oil (2ml/kg), Group C: Fed with un-oxidized oil (2ml/kg), Group D: Fed with green tea (200 mg/kg), Group E: Fed with green tea (100 mg/kg), Group F: Fed with 100 mg/kg green tea along with oxidized oil (2ml/kg), Group G: Fed with 200 mg/kg green tea along with oxidized oil (2ml/kg), Group H: Fed with green tea along with oxidized oil (2ml/kg). Feeding was carried out regularly for twenty-one days.

Hematological and Serum BiochemicalParameters

About 5ml of whole blood samples were collected on days zero, 11th, and 21st of the experiment for hematological parameters. About 2 ml of whole blood was used to analyze Hemoglobin count (Hb), Platelet count (P), White blood cells (WBCs), Neutrophils (N), Lymphocytes (L), Monocytes (M), Eosinophils (E), and Basophils (B) using a fully automated blood hematology analyzer. The 3ml blood was collected in gel tubes, and serum was used to analyze total Triglyceride (Tg), total cholesterol (C), Blood Sugar (S), SGPT (ALT), and Creatinine, Urea, HDL, and LDL.

Liver Histopathology

At the end of the experiment, one rabbit from each group was slaughtered, and the liver samples were collected and preserved in formalin buffer (10%). Tissue sectioning were made, and stained and histopathological examinations were done as described (Zeb and Ullah 2015). The prepared slides were observed under the light microscope, model no. M 7000 D (SWIFT, Japan), and the pictures were taken by a digital camera mounted on a microscope with a resolution of 2.4 MP.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) and Tukey test using online statistical software, GraphPad Prism Demo Version 05 (www.graphpad.com). Data were presented as a mean with a standard deviation of n=3 triplicate. The mean and standard deviation were sorted out for each parameter.

RESULTS

The whole blood and serum samples were used to analyze hematological and biochemical parameters. Histopathology of the liver was done. The results have been presented as follows.

Hematological Parameters

On day zero, whole blood was collected from all the rabbits; the results were in the normal reference range, as shown in figure 1. A significant alteration ($P<0.05$) was observed in the mean values of all hematological parameters on day 11th, as shown in figure 2. The mean values of all the hematological parameters were significantly changed in the group fed with oxidized oil. The un-oxidized oil and green tea combined showed beneficial effects on these parameters when fed to rabbits alone. On day 21st, the mean values were significantly changed in the rabbits fed with oxidized oil. The green tea improved the altered hematological parameters when provided with oxidized oil, as shown in figure 3.

Serum Biochemical Parameters

The blood samples were collected in gel tubes, and serum was isolated to analyze biochemical parameters. The mean values of all the serum biochemical parameters were almost in the normal reference range, as shown in figure no 1. On day 11th, the results showed significant changes ($P<0.05$) between the mean values of the parameters among the different groups. The oxidized oil significantly raised the SGPT values and altered the lipid profile. The positive effects of the green tea alone or in combination and un-oxidized oil were observed on the mean values of biochemical parameters (Figure 2). A significant elevation was recorded in blood glucose level, urea, triglycerides, LDL, and a decrease in HDL values in the rabbits fed with oxidized oil. There was no significant difference ($P<0.05$) in the mean values of different parameters between the rabbits fed with oxidized oil alone or green tea.

The mean values of all the serum biochemical parameters were significantly altered on day 21st in the group fed with oxidized oil. Green tea had a positive effect on the improvement of the altered parameters. The beneficial effect of the un-oxidized oil was observed on the serum biochemical parameters (Figure 3).

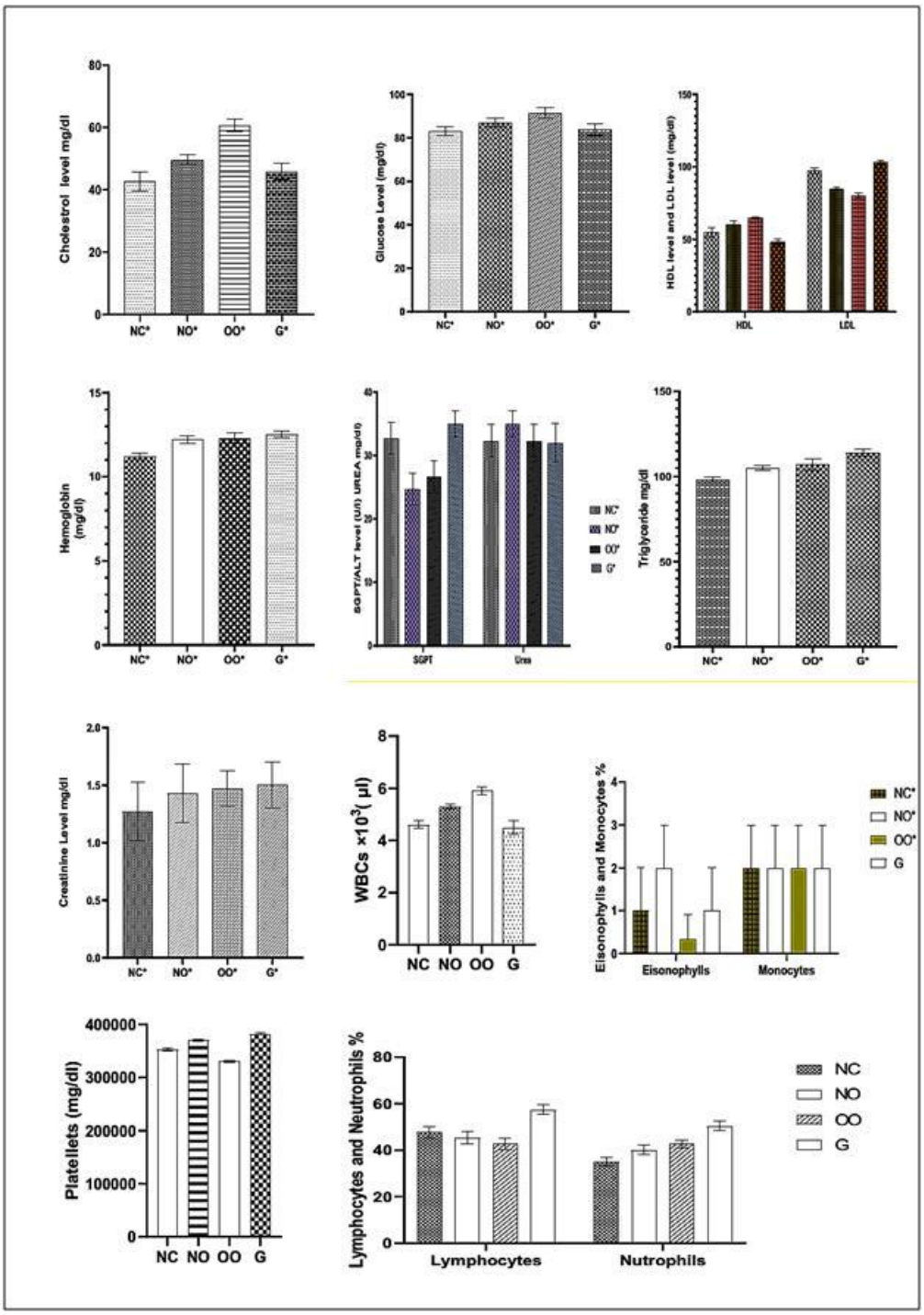

Figure 1:Hematological and Biochemical Parameters of Rabbits Different Groups at Day Zero. Same alphabets in a row show no significant difference (P<0.05), while different alphabets in the same row show a significant difference (P<0.05)

A: Fed with regular diet, B: Fed with oxidized oil (2ml/kg), C: Fed with un-oxidized oil (2ml/kg), D: Fed with green tea (200 mg/kg).

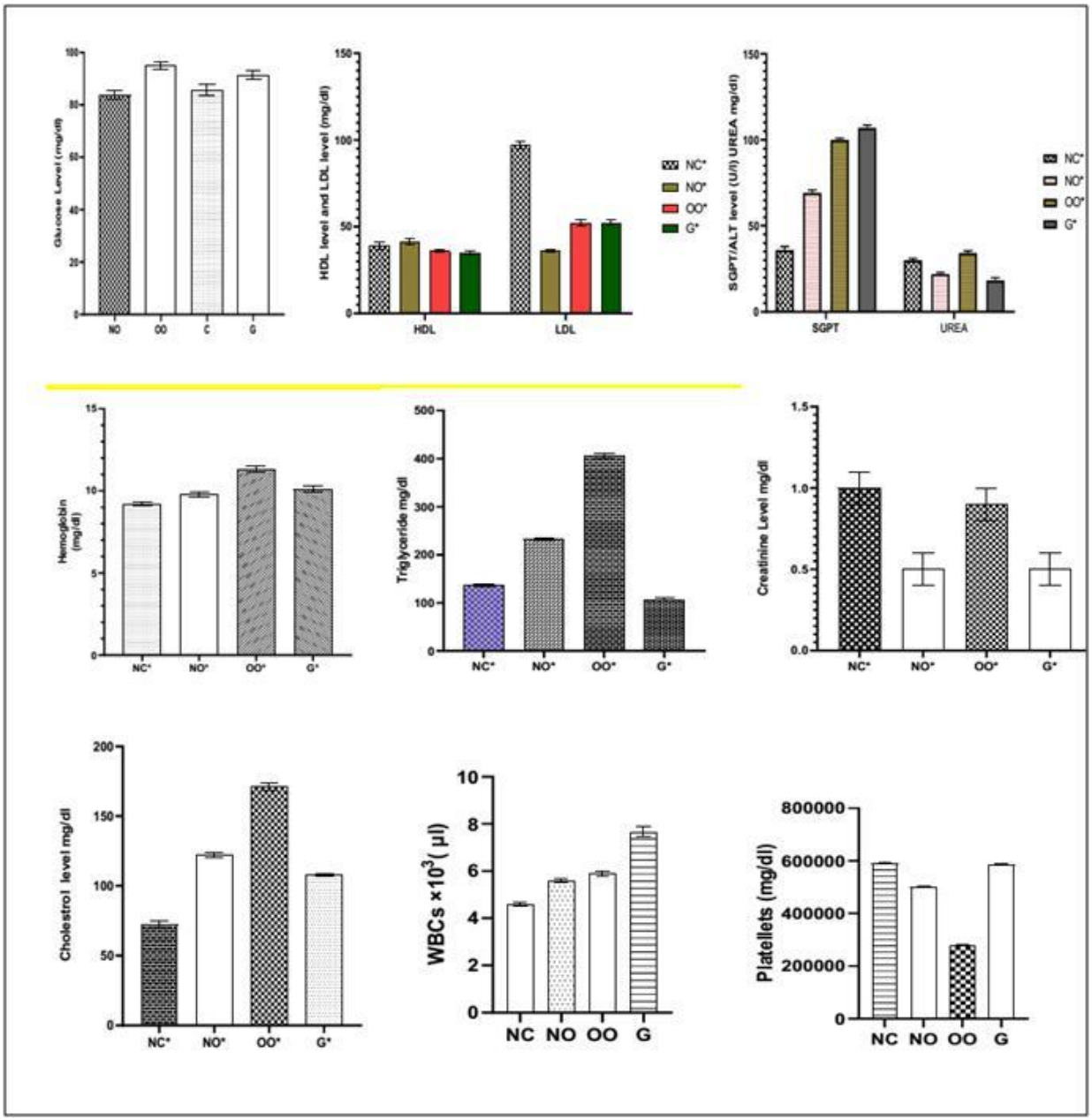

Figure 2: Hematological and Biochemical Parameters of Rabbits Different Groups at Eleventh Day. Same alphabets in a row show no significant difference (P<0.05), while different alphabets in the same row show a significant difference (P<0.05)

A: Fed with regular diet, B: Fed with oxidized oil (2ml/kg), C: Fed with un-oxidized oil (2ml/kg), D: Fed with green tea (200 mg/kg).

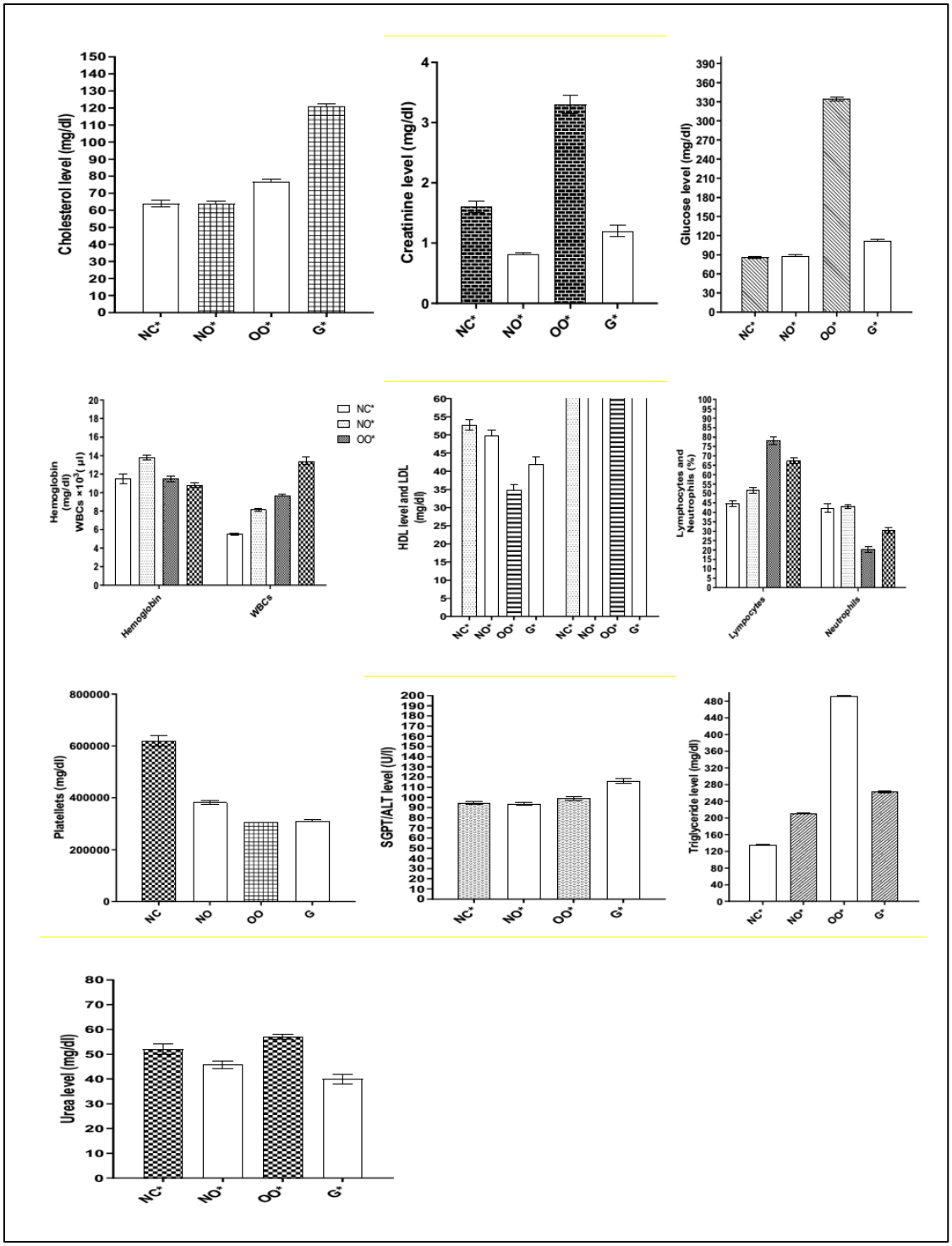

Figure 3: Hematological and Biochemical Parameters of Rabbits Different Groups at 21 Days. Same alphabets in a row show no significant difference (P<0.05), while different alphabets in the same row show a significant difference (P<0.05)
A: Fed with a regular diet, B: Fed with oxidized oil (2ml/kg), C: Fed with un-oxidized oil (2ml/kg), D: Fed with green tea (200 mg/kg).

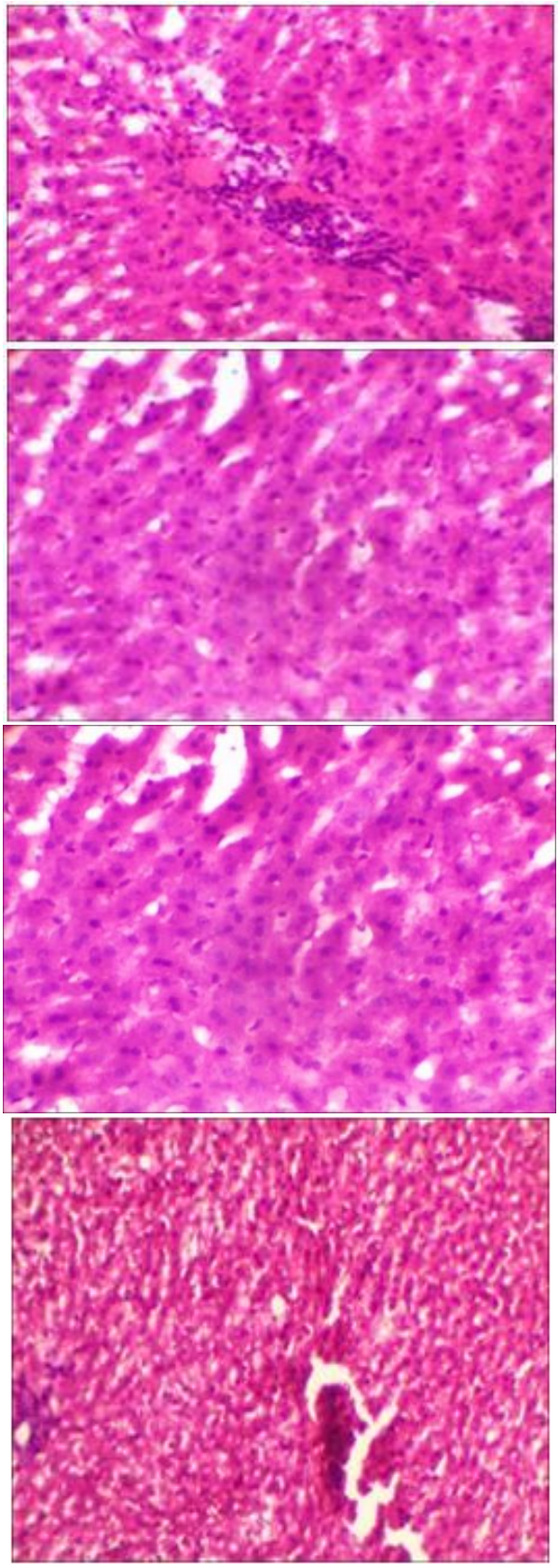

Figure 4: Histopathology of the rabbits' liver samples
A: Rabbits fed with a regular diet, B: Rabbits fed with un-oxidized sunflower oil (2ml/kg body weight), C: Rabbits fed with oxidized sunflower oil (2ml/kg body weight), D: Rabbits fed with green tea (200 mg/kg body weight)

Histopathological Examination

Histopathology of the liver obtained from all the groups was done to check the abnormalities at the tissue level. The liver of the control rabbits showed typical morphology and normal endothelial linings of focal veins without pericentral fibrosis and fatty changes. The Kupffer cells were non-reactive. The texture of the hepatic line was well indeed (Figure 4A). In un-oxidized oil fed group, there was mild necrosis, focal vein congestion, and fatty changes (Figure 4B). In the rabbits provided with oxidized oil, the liver histology showed cholestasis zone 3 necrosis with mild fatty change. The hepatocytes showed the vesicular fatty changes (Figure 4C). Vacuolization, and mild necrosis was seen in green tea (200mg/kg) fed group (Figure 4D).

DISCUSSION

Heating at a high temperature modifies sunflower oil's quality and alters its quality (Gertz et al. 2000), color, and chemical properties (Zeb and Mehmood 2012). Heating oil produces toxic chemicals that may affect health (Tyagi and Vasishtha 1996). In the present study, we heated sunflower oil at 100 oC for 5 hours and fed to rabbits alone or in combination with green tea at different dosage levels. The green tea was also provided independently or in combination to rabbits to determine its effects on other biochemical and hematological indices. Histopathology was done for further confirmation of abnormalities at the tissue level.

In the present study, we observed an increase in white blood cells (WBCs), Hemoglobin (Hb) concentration, and neutrophils (N) count while a decrease in platelets count, lymphocytes (L), and monocytes after feeding with oxidized oil when compared with control. When green tea was provided in high dose and oxidized oil, significant changes were recorded. Oxidized olive oil alone was fed to rats or in combination with α-tocopherol, and the hematological parameters were altered by oxidized olive oil while α-tocopherol normalized the modified parameters (Zeb and Khan 2019). Oxidized Corn oil was fed to rabbits, and decreased total red blood cells and hemoglobin and increased WBCs count was documented (Ul 2015). We found average values of glucose, ALT, and urea level, serum cholesterol, triglycerides, HDL-c, and low LDL in un-oxidized oil-fed rabbits. These values were significantly affected by oxidized sunflower oil. A significant effect was recorded when green tea was fed alone or combined with oxidized sunflower oil to rabbits.

When the edible oils are heated, they become toxic and, if fed to subject animals, raise the liver enzymes (Shastry et al. 2011), confirming the hepatotoxic effects of oxidized lipids. At the same time, Oleuropein is present in un-oxidized oils and prevents hepatocytes damage. Oxidized fats cause oxidative stress, causing liver injury, increasing the ALT level [15][12], and increasing the total serum cholesterol, triglycerides, LDL, glucose, and ALT. In contrast, serum HDL was significantly decreased when oxidized olive oil was fed to rats. Many polar TAGs are formed when oils are thermally oxidized (Frankel 2010), and consumption of these polar TAGs significantly affect serum biochemical parameters. Oxidized fats in food are absorbed and cause atherosclerosis by increasing blood cholesterol levels (Staprãns et al. 1996).

The liver of rabbits fed with a regular diet showed normal morphology with no evidence of fatty changes. A mild fatty liver with zone 2 necrosis was found in un-oxidized sunflower oil-fed rabbits, while cholestasis and zone 3 necrosis with mild fatty change were observed in the oxidized oil-fed rabbits. Mild changes were observed in the green tea-fed rabbits alone or combination. High lipid contents in the diet caused a decrease in hepatic superoxide dismutase, catalase and glutathione peroxidase and increased lipid peroxidation. Liver histology confirmed necrosis and hepatic vacuolation(Dhibi et al. 2011).

CONCLUSION

It has been concluded that oxidized sunflower oil significantly alters the hematology and serum biochemical parameters and affects the liver compared to un-oxidized oil. Green tea has anti-oxidant potential and minimizes the toxic effects of oxidized fat.

CONFLICT OF INTEREST

The authors declared that the present study was performed without conflict of interest.

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AUTHOR CONTRIBUTIONS

SK and FI performed animal treatment, analyzed hematological and biochemical parameters, and wrote the manuscript, and the AAK designed the study and reviewed the manuscript. GYZ designed the graphs, analyzed the results, and reviewed the manuscript. All authors read and approved the final version.

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