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Regular paper

Assessing the pharmacological and biochemical effects of *Salvia hispanica* (Chia seed) against oxidized *Helianthus annuus* (sunflower) oil in selected animals

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Oil oxidation is important in terms of taste, nutritive component quality and toxic effect of the oil. In this study, the oxidized sunflower oil was used along with chia seed in rabbits for the determination of its effects on various hematological and serum biochemical parameters as well as on liver histopathology. Three rabbits were fed with oxidized oil (obtained by heating) at the dose rate of 2 ml/kg body weight by mixing it with green fodder. The other rabbit groups were fed with Chia seed at dose rate of 1, 2 and 3 g/kg along with oxidized sunflower oil. Chia seed was fed alone to three rabbits at the dose rate of 2 g/kg body weight. All rabbits were fed regularly for twenty-one days. For the determination of hematological and biochemical parameters, whole blood and serum samples were collected on different days during feeding period. For histopathology, liver samples were used. Significant changes ($p<0.05$) were noted in the hematology and biochemical indices in the rabbits that were fed with oxidized sunflower oil alone, and along with different doses of Chia seed. In a dose-dependent manner, all these parameters were significantly improved ($p<0.05$), when the amount of Chia seed was increased. The biochemical and hematological indices were in normal range in the group fed only with Chia seed. In oxidized oil fed group, liver histopathological analysis showed that cholestasis was present at both sides (bile pigment secretion) and zone 3 necrosis with mild inflammatory cells. Mild vacuolization of hepatocytes was also observed. In Chia seed fed group, hepatocyte vacuolization and mild necrosis was noted. It was concluded that oxidized sunflower oil alters the biochemical and hematological parameters and causes liver abnormalities. Chia seeds act as an antioxidant and retrieve those alterations.

Keywords: Chia seed, hematological parameters, oxidized sunflower oil, rabbits

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Abbreviations: ALT, alanine transaminase; B, basophils; C, total cholesterol; E, eosinophils; H, hemoglobin; HDL, high density lipoproteins; L, lymphocytes; LDL, low density lipoproteins; M, monocytes; N, neutrophils; P, platelets; S, blood glucose; TG, triglycerides; WBCs, white blood cells

INTRODUCTION

Vegetable oils and fats are important constituents of foods and are essential components of our daily diet (Brahmi *et al*., 2020). Vegetable oils are obtained by mechanical expelling or solvent extraction of oleaginous seeds (soybeans, rapeseed, sunflower, etc.) or oleaginous fruit like palm and olive (Vidrih *et al*., 2010). Vegetable oils generally contain triglycerides (about 98 g/100 g) (Qian *et al*., 2020). Triglyceride is formed from a reaction between glycerol and fatty acids and other substances in a small proportion (Gnanaprakasam *et al*., 2021). Some of them such as diglycerides, vitamins, phytosterols, tocopherols and polyphenols have important health benefits in humans (Gharby *et al*., 2021; Chew *et al*., 2016), and therefore, they should not be removed during processing. Other compounds known for their negative effect on the quality and stability of oils include free fatty acids, unsaponifiable matters, waxes, pigments, solid impurities (mainly fibers), oxidation products (peroxides, aldehydes, ketones, alcohols, and oxidized fatty acids) (Gharby *et al*., 2016; Aliyar-Zanjani *et al*., 2019; Said *et al*., 2022). Several plants contain different chemicals which can be used for treatment of various diseases if they are consumed entirely or in parts with lower cost and less side effects (Sana *et al*., 2022; Bisht *et al*., 2021). Sunflower oil is one of the most widely grown essential oils in the world. The total world's oilseed production is forecasted at nearly 647 million tons (United States Department of Agriculture Foreign Agricultural Service Oilseeds: World Markets and Trade. 2022). In 1998, the world's seed oil was about 28.5 million tons and, as a vegetable oil, only soybean (*Glycine max*) and *Brassica* species (*Brassica napus* and *Brassica campestris*) produced more oil (Flagella *et al*., 2002). Sunflower (*Helianthus annuus*) is used in food, for oil, as a dye, for medicinal purposes, and as an ornamental plant species. Sunflower oil has been used since ancient times as a food and as a medicinal plant to cure many ailments. From a dietary point of view, a diet enriched in monounsaturated fatty acids has been recommended to reduce cholesterol in blood plasma (Dimitrijevic and Horn 2018). Sunflower is often used to produce oils from seeds but is also used as a protein source for human consumption as well as food (Choe and Min 2006). Oil oxidation is important in terms of palatability, nutritional quality and acidity of edible oils. Various chemical compounds, autoxidation and photosensitized oxidation are responsible for the degra-

dation of edible oils during production and storage with respect to oxygen (Khan *et al*., 2022; Knez *et al*., 2019).

Salvia hispanica, also called Chia, is an annual herbaceous plant native to southern Mexico and northern Guatemala. It belongs to *Lamiales,* family Labiatae, subfamily Nepetoideae, and genus *Salvia* (Segura-Campos *et al*., 2014). The *Salvia* genus comprises of about 900 species, which have been extensively distributed for thousands of years in many regions of the world, including South Africa, North, Central and South America, and Southeast Asia (Grancieri *et al*., 2019; Ullah *et al*., 2016; Campos *et al*., 2016; Das *et al*., 2018; Mohd Ali *et al*., 2012).

Many studies have reported that Chia today is grown not only in Mexico and Guatemala, but also in Australia, Bolivia, Columbia, Peru, Argentina, America, and Europe. Today, Mexico is known as the largest producer of Chia in the world (Silva *et al*., 2016). Chia is the dietary seed of *Salvia hispanica*, a flowering plant, known for its antioxidants that is often used in food production (Coorey *et al* 2016). Recently, Chia seeds have been given more consideration and have become one of the most popular foods in the world based on their medicinal values and nutritional properties (Ullah *et al*., 2016; Das *et al*., 2018; Mohd Ali *et al*., 2012; Silva *et al*., 2016). Coorey *et al.* (2016) demonstrated that Chia is an excellent food ingredient as it contains a huge amount of α-linolenic acid and is easily added to commercial foods. In addition to that it has also been reported that Chia seeds contain high percentage of fatty acids, which make it crucial for health, antioxidant, and antimicrobial property (Ullah *et al*., 2016; Mohd Ali *et al*., 2012; Ixtaina *et al*., 2008; Reyes *et al*., 2008). Furthermore, several other studies (Grancieri *et al*., 2019; Silva *et al*., 2016; Ixtaina *et al*., 2008; Reyes *et al*., 2008; Ayerza *et al*., 2016; Muñoz *et al*., 2012) demonstrated that the Chia is an oil seed composed of fats, carbohydrates, dietary fiber, proteins, vitamins (A, B, K, E, D), minerals and antioxidants and its benefits of using as a nutritional supplement are numerous, such as supporting digestive system, helping the intestinal mucosa, stronger bones, reducing the risk of constipation, irritable bowel disease, heart diseases, diabetes, and many more (Silva *et al*., 2016; Correy *et al*., 2016; Ixtaina *et al*., 2008; Reyes *et al*., 2008; Ayerza *et al*., 2016; Muñoz *et al*., 2012; de Falco *et al*., 2018). In the region of Malakand, Khyber Pakhtunkhwa, Pakistan, different food items are fried and cooked using either ghee or oils. Persistent heating causes ghee and oil oxidation, hence, making it toxic. Therefore, the present study was aimed to check the toxic effects of oxidized sunflower (*Helianthus annus*) oil on hematological and biochemical parameters in rabbits. As Chia seeds (*Salvia hispanica*) are sources of one of the potent antioxidants, they were used to check its curing potential against the toxic effects of oxidized sunflower (*Helianthus annuus*) oil.

MATERIALS AND METHODS

Materials

Sunflower oil was purchased from local market of Matta, Swat, Khyber Pakhtunkhwa, Pakistan. The sunflower oil was selected based on its high linoleic acid and oleic acid content. Chia seeds and rabbits (n=60) were purchased in the local market of Chakdara, Lower Dir, Khyber Pakhtunkhwa. EDTA containing tubes and gel tubes were purchased from the local market for whole blood collection and serum isolation. Formaldehyde and chloroform were provided by organic chemistry laboratory, Department of Chemistry, University of Malakand.

Thermal oxidation of oil

Sunflower oil samples were subjected to a five-hour long regular heating on hot plates at 100°C. These samples were then kept at –20°C to prevent them from further chemical changes.

Experimental animal clustering and feeding

Rabbits were reared in Bio-park of University of Malakand. Food and water were freely available to all the animals. They had an average weight of 1200 grams at the start of the experiment, and among the 60 rabbits, 21 individuals were selected for the experiment based on body weight and health status. The study was started after the approval of the ethical committee, Department of Biotechnology, University of Malakand. Rabbits were divided into seven groups, each having three rabbits (n=3). Groups were labelled as NC, NO, OO, C, CO1, CO2 and CO3 representing negative control, normal sunflower oil, oxidized oil, Chia seed only, Chia seed with oxidized oil (low dose), Chia seed with oxidized oil (medium dose) and Chia seed with oxidized oil (high dose), respectively. The negative control group was fed with green fodder and water. NO was fed with normal sunflower oil at a dose of 2 ml/kg with fodder and water. Group OO was fed with oxidized sunflower oil at a dose of 2 ml/kg. Group C was given Chia seed at a dose of 2 g/kg. Group CO1 was fed with Chia seed at a dose of 1g/kg and oxidized sunflower oil at a dose of 2 ml/kg. Group CO2 was fed with Chia seed at a dose of 2 g/kg and oxidized sunflower oil at a dose of 2 ml/kg. Group CO3 was fed with Chia seed at a dose of 3 g/kg and oxidized sunflower oil at a dose of 2 ml/kg. The feeding was continued for 21 consecutive days and blood samples were collected at day 0, 11 and 21 for hematological parameters and serum biochemical parameters.

Hematological and serum biochemical parameters

The whole blood was used for the analyses of hemoglobin (Hb), platelets (P), white blood cells (WBCs), neutrophils (N), lymphocytes (L), monocytes (M), eosinophils (E) and basophils (B) count by using a fully automated blood hematology analyzer (ERBA – XL 1000). About 5 ml of blood was collected and transferred to gel tubes to isolate serum. The serum was used for the analyses of total triglyceride (TG), total cholesterol (C), blood glucose (S), alanine transferase (ALT), creatinine, urea, high density (HDL) and low density lipoproteins (LDL) level.

Histopathological examination of liver

At the end of the experiment, rabbits were slaughtered according to the method described by Hussain and others (Hussain *et al*., 2022) and their liver was isolated and preserved in formalin buffer (10%). Tissues sectioning were made, stained and histopathologically examined as described by Khan and others (Khan *et al*., 2022). Prepared slides were observed under the light microscope, model no. M 7000 D (SWIFT, Japan) and images were taken by a digital camera coupled with a microscope with a resolution of 2.4 Mpixel.

Table 1. Effect of oxidized sunflower oil and Chia seed on different hematological parameters in rabbits on different days.

Groups	Hemoglobin (gm/dl)			Platelets (x10^9/L)			Neutrophils (%)			Lymphocytes (%)			Monocytes (%)			Eosinophils (%)		
	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21
NC	8.37± 0.35± a	11.2± 2.00a	12.2± 1.6a	5.5×105± 300a	5.8×105± 200a	3.8×10^5± 200a	42.3± 2.52a	53.0± 2.00a	40.0± 2.00a	42.0± 2.00a	50.7± 2.52a	55.0± 2.0a	1.67± 0.57a	1.00± 1.00a	2.33± 1.3a	1.00± 1.00a	3.00± 2.00a	3.00± 2.0a
NO	7.47± 0.45a	12.0± 2.15a	14.3± 1.8a	5.5×105± 251a	5.0×105± 200b	3.9×10^5± 251b	42.7± 2.52a	67.0± 2.00b	42.7± 2.52a	41.7± 2.52a	34.0± 2.00b	52.0± 2.00a	2.00± 1.00a	1.00± 1.00a	3.67± 1.53a	2.00± 1.00a	2.33± 1.53a	2.67± 1.53a
OO	9.33± 0.35b	11.3± 2.15a	13.4± 2.1a	5.5×105± 251a	2.7×105± 200c	3.0×10^5± 251c	41.3± 2.52a	42.3± 2.52c	22.7± 2.2b	43.0± 2.00a	59.0± 2.00c	78.0± 2.0b	1.00± 1.00a	2.00± 1.00a	2.33± 1.5a	1.00± 1.00a	3.00± 2.00a	3.00± 2.0a
C	10.7± 0.20c	13.2± 2.15a	13.7± 2.4a	5.4×105± 300b	3.8×105± 251d	3.5×10^5± 200d	45.0± 2.00a	98.0± 2.00d	32.0± 2.00b	45.0± 2.00a	1.00± 1.00d	67.0± 2.00c	2.00± 1.00a	2.00± 1.00a	2.33± 1.53a	1.33± 0.57a	1.33± 1.53a	4.00± 2.65a
CO1	7.40± 0.40a	12.4± 1.90a	12.4± 1.5a	5.6×105± 200a	4.2×105± 200e	2.8×10^5± 305e	47.7± 2.52a	97.3± 2.52d	26.0± 2.0b	42.0± 2.00a	1.00± 1.00d	65.7± 2.2c	2.00± 1.00a	1.33± 0.577a	4.00± 2.0a	1.00± 1.00a	2.33± 1.53a	4.00± 2.0a
CO2	11.3± 0.25c	10.6± 1.80a	12.4± 2.1a	5.5×105± 251a	5.7×105± 200f	4.0×10^5± 251f	48.7± 3.06a	83.0± 2.00e	29.0± 2.0b	43.7± 2.52a	18.0± 2.00e	68.0± 2.0c	1.00± 1.00a	2.00± 1.00a	2.33± 1.5a	1.33± 0.57a	3.00± 2.00a	2.67± 1.3a
CO3	12.3± 0.30d	12.5± 2.00a	11.5± 1.8a	5.5×105± 300a	2.5×105± 200g	2.0×10^5± 200g	47.7± 8.50a	66.7± 2.52b	31.3± 2.52b	41.0± 2.00a	31.7± 1.53b	68.3± 2.52c	2.00± 1.00a	2.67± 1.53a	3.33± 1.53a	1.00± 1.00a	2.00± 2.00a	3.33± 1.53a

Same letters in a row show no significant difference ($p<0.05$), while diverse letters in the same row indicate for a significant difference ($p<0.05$).NO-normal Oil, OO – oxidized oil, C – Chia Seed, CO1– Chia seed at a dose of 1 g/kg along with 2 ml of oil, CO2 – Chia seed at a dose of 2 g/kg along with 2 ml of oil, CO3 – Chia seed at a dose of 3 g/kg along with 2 ml of oil

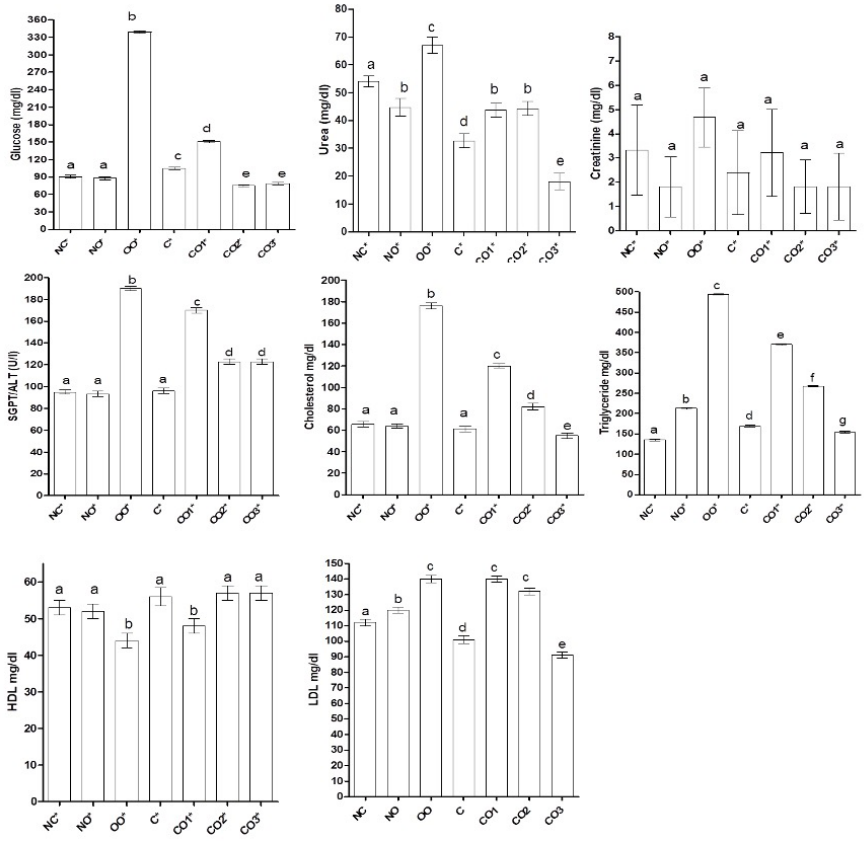

Figure 1. Effects of oxidized sunflower oil and Chia seed on different biochemical parameters in rabbits.

Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) and Tukey test using online statistical software, prism demo version 05 (www.graphpad.com). Data were presented as mean from triplicate results (n=3) with standard deviation. The mean and standard deviation were sorted out for each parameter.

RESULTS

Effects on hematological parameters

Results showed no significant effect (p<0.05) on the Hb level; however, the platelet count remarkably decreased in all groups after the 21st day. The platelet count was the lowest in the CO3 group. Similarly, no significant change was observed for neutrophils and lymphocyte count. The monocyte count doubled in both CO2 and CO3 groups. The eosinophile number increased in all groups, but such increase was not remarkably high. The results from all measurements (day 0, 11th and 21st day) were combined in Table 1. Different biochemical parameters of the rabbits were shown in Fig. 1.

Effects on biochemical parameters

The glucose level was significantly (p<0.05) high in the oxidized oil (OO) group; however, groups C and CO1 also reflected substantial elevated sugar level. The urea level remained the same in all groups except for CO3 that showed a significant decrease at 21st day. The SGPT/ALT level were significantly (p<0.05) increased in all groups, especially among OO, C, CO1, CO2, and CO3 variants. After the 21st day, the cholesterol levels in NC, NO and CO2 groups remained almost like day 0 values. However, in the OO group, the cholesterol level was high, and the result was the opposite in the C group, wherein cholesterol levels decreased. In CO1 variant, the total cholesterol level significantly (p<0.05) increased, as compared to CO3, where the cholesterol level decreased on the 21st day. The triglyceride values were significantly (p<0.05) elevated in all groups, but they were remarkably high in OO, CO1, and CO2 variants. The HDL levels in all groups showed no significant change. The LDL levels increased in all groups except CO3 wherein, a significant decrease was noted; pertinent to mention, the OO group showed the most negatively correlated LDL values. The results of biochemical parameters on days 0, 11 and 21 were combined in Table 2.

Histopathological pattern

At the end of the experiment, the rabbits were slaughtered, and liver samples were collected for the histopathological analysis. Liver slides were studied under 10X and 40X magnitude for detailed histopathological changes. Results have been presented in Fig 2. Liver histopathology of control variant showed that endothelial linings of central veins had normal morphology, and no evidence of pericentral fibrosis was noted. Kupffer cells were non-reactive. The orientation of the hepatic cord was very good. Hepatic portal veins and arteries showed a normal structure (Fig 2A). On the contrary, in the oxidized oil fed group, cholestasis was present on both sides (bile pigment secretion). Zone 3 necrosis with mild inflammatory cells was noted. Mild hepatocytic vacuolization was also observed (Fig 2B). However, in the normal oil fed group, there was mild zone 2 necro-

Table 2. Effect of oxidized sunflower oil and Chia seed on different biochemical parameters in rabbits on different days.

Groups	Glucose level mg/dl			Urea level mg/dl			creatinine level (mg/dl)			SGPT/ALT level (U/l)			Cholesterol level (mg/dl)			Triglyceride level (mg/dl)			HDL level (mg/dl)			LDL level (mg/dl)		
	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21
NC	77.0 ± 2.0a	63.0 ± 2.00a	90.7 ± 2.52a	35.0 ± 2.0a	33.0 ± 2.00a	54.0 ± 2.00a	0.60 ± 0.2a	1.21 ± 0.20a	3.33 ± 1.86a	34.3 ± 2.5a	40.3 ± 2.52a	95.0 ± 2.00a	67.0 ± 2.0a	125 ± 2.08a	65.7 ± 2.52a	120 ± 2.00a	145 ± 2.00a	135 ± 2.00a	54.7 ± 3.0a	39.0 ± 2.00a	53.0 ± 2.00a	97 ± 2.00a	97.0 ± 2.00a	112 ± 2.00a
NO	77.0 ± 2.00a	85.7 ± 1.53b	87.7 ± 2.52a	33.7 ± 2.08a	23.3 ± 2.52b	44.7 ± 3.21b	0.833 ± 0.15a	0.700 ± 0.20a	1.81 ± 1.24a	34.0 ± 2.00a	71.0 ± 2.00b	93.3 ± 2.52a	65.0 ± 2.00a	121 ± 2.00a	64.0 ± 2.00a	125 ± 2.52a	233 ± 2.00b	213 ± 2.52b	60.3 ± 2.52b	43.0 ± 2.00a	52.0 ± 2.00a	85 ± 1.00b	37.0 ± 2.00b	120 ± 2.00a
OO	82.7 ± 2.52b	94.7 ± 2.52c	339 ± 2.00b	36.3 ± 2.52a	35.0 ± 2.00a	67.0 ± 3.00c	0.833 ± 0.20a	0.86 ± 0.15a	4.67 ± 1.22a	39.3 ± 2.52b	100 ± 2.52c	190 ± 2.00b	73.7 ± 2.52b	170 ± 2.00b	176 ± 3.06b	126 ± 2.52a	410 ± 2.00c	494 ± 2.00c	64.7 ± 0.58b	38.3 ± 2.52a	44.0 ± 2.00b	80.3 ± 1.53b	51.7 ± 2.52c	140 ± 2.52b
C	85.7 ± 1.53b	90.7 ± 2.52c	105 ± 2.08c	38.0 ± 2.00a	23.3 ± 1.53b	32.7 ± 2.52d	1.03 ± 0.252a	0.83 ± 0.15a	2.40 ± 1.73a	38.7 ± 2.52b	94.7 ± 2.52c	96.3 ± 2.52a	75.0 ± 2.00b	111 ± 2.52c	61.3 ± 2.52a	131 ± 2.00b	113 ± 2.00d	169 ± 2.00d	75.0 ± 1.00c	41.0 ± 2.00a	56.3 ± 2.52a	95.3 ± 1.53a	49.3 ± 2.52d	101 ± 2.52c
CO1	73.0 ± 2.00a	82.3 ± 2.52b	151 ± 2.00d	28.7 ± 2.52a	35.3 ± 2.52a	43.7 ± 2.52b	0.967 ± 0.30a	0.73 ± 0.25a	3.22 ± 1.79a	41.3 ± 2.08b	81.7 ± 2.52d	170 ± 2.52c	71.7 ± 2.52b	148 ± 2.65	120 ± 2.00c	132 ± 3.51b	554 ± 2.00e	370 ± 2.00e	48.0 ± 2.00d	39.0 ± 2.00a	48.0 ± 2.00b	103 ± 1.53c	51.0 ± 2.00c	140 ± 2.00b
CO2	93.7 ± 2.52c	101 ± 2.08d	75.0 ± 2.00e	33.0 ± 2.00a	41.0 ± 2.00c	44.3 ± 2.52b	1.33 ± 0.35b	0.83 ± 0.25a	1.82 ± 1.11a	32.7 ± 2.52a	54.3 ± 1.15e	123 ± 2.52d	79.0 ±1.73b	111 ± 2.52c	82 ± 3.06d	136 ± 2.52b	136 ± 2.00f	267 ± 2.00f	63.0 ± 1.00b	40.3 ± 2.52a	57.0 ± 2.00a	108 ± 1.53c	48.3 ± 2.52d	132 ± 2.00d
CO3	94.7 ± 2.52c	143 ± 3.06e	79.0 ± 2.00e	35.3 ± 2.08a	38.0 ± 2.65d	18.0 ± 3.00e	1.23 ± 0.30a	0.94 ± 0.26a	1.81 ± 1.38a	38.0 ± 2.00b	83.7 ± 1.53d	123 ± 2.52d	77.0 ± 2.00b	123 ± 2.00a	55 ± 2.52e	135 ± 2.52b	111 ± 2.00d	154 ± 2.52g	61.0 ± 2.00b	38.0 ± 2.00a	57.0 ± 2.00a	110 ± 2.52d	95.0 ± 2.00a	91 ± 2.00c

Same letters in a row show no significant difference ($p<0.05$) while diverse letters in the same row indicate for a significant difference ($p<0.05$). NO-normal oil, OO – oxidized oil, C – Chia Seed, CO1 – Chia seed at a dose of 1 g/kg along with 2 ml of oil, CO2 – Chia seed at a dose of 2 g/kg along with 2 ml of oil, CO3 – Chia seed at a dose of 3 g/kg along with 2 ml of oil

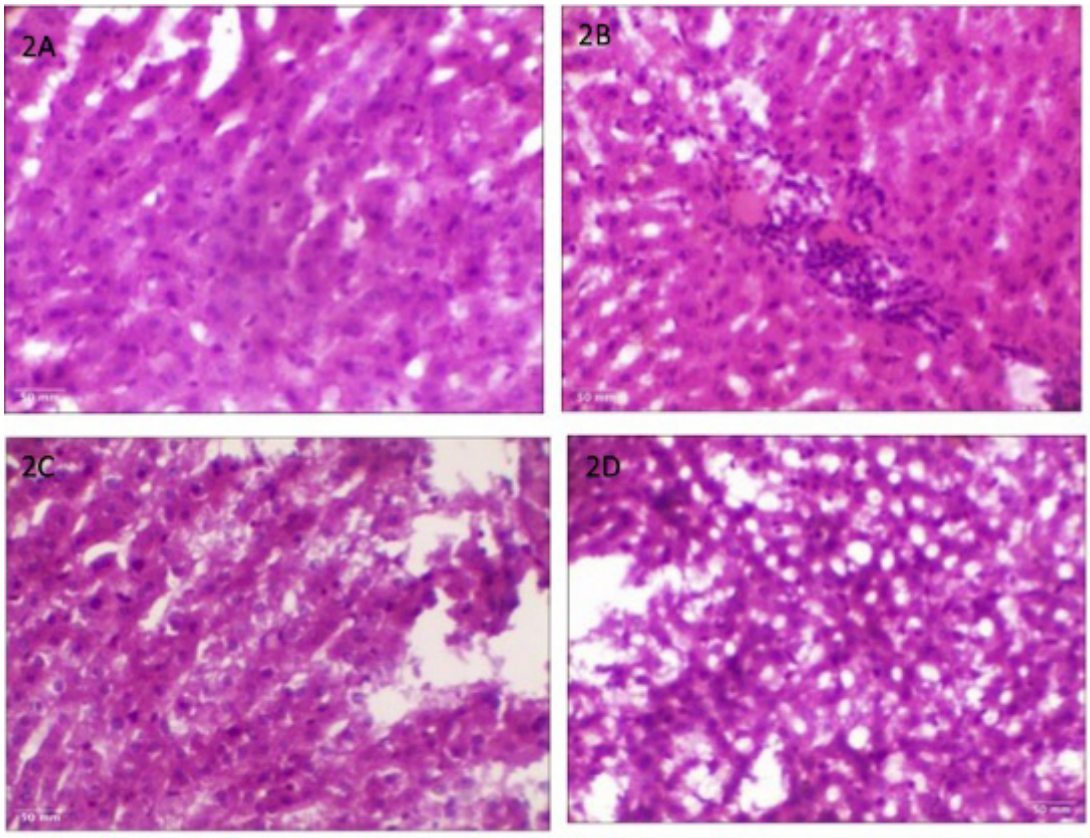

Figure 2. Histopathology of the normal rabbit liver (2A), oxidized oil-fed rabbits (2B), normal oil (2C) and Chia seed-fed rabbits (2D).

sis especially central vein inflammation and fatty changes (Fig 2C). In Chia seed fed group, there was hepatocyte vacuolization and visible signs of mild necrosis (Fig 2D).

DISCUSSION

It is common practice to repeatedly heat vegetable oils at high temperatures during cooking. Oils are heated during food preparation or deep frying. The present study aimed to sort out the effect of Chia seed against oxidized sunflower oil in rabbits. In our findings, lymphocyte count was significantly increased, while there was a low number of platelets, neutrophils, and monocytes after feeding with oxidized oil as compared to control. The results for these parameters were similar to control ones when fed with Chia seeds. Similar to our results, fresh palm oil was fed to rats, and it was observed that heated oil decreased PCV, Hb level, RBCs and increased WBCs (Mesembe *et al*., 2004). Similarly, oxidized olive oil significantly altered hematological parameters in rats (Khan *et al*., 2017). No effects of repeatedly heating cooking oil were observed on hematological parameters after its administration to Wistar rats (Shue *et al*., 1968; Perumalla *et al*., 2016), which is not in accordance with our findings. In our study, we heated the sunflower oil for 5 hours at 100^{o}C which may have led to the accumulation of free radicals and altered the hematological parameters. The consumption of Chia has shown good digestibility, hypoglycemic effects, improved lipid and glycemic profiles, and reduced fat deposition in the animal liver.

There was a statistically significant increase in creatinine and urea level in rabbits that were fed with oxidized sunflower oil, and the Chia seed reduced their levels to normal. The co-administration of oxidized oil and Chia seed decreased the creatinine and urea level in a dose-dependent manner. Much of the toxicity of severely heated food oils has been associated with a non-urea-adducting fatty acid (urea filtrate) fraction (Shue *et al*., 1968; Billek *et al*., 2000). Certain fractions of the heated oils, the total polar materials cause growth retardation, increased liver and kidney weights and disorders of the enzyme system, but only if fed in high doses (Billek *et al*., 2000; Ani *et al*., 2015).

The significant increase in serum creatinine concentration in the group fed with thermo-oxidized palm oil may be suggestive of possible renal system damage. Creatinine levels in plasma are usually measured to determine acute or chronic renal insufficiency. They are usually raised in renal disease (Ani *et al*., 2015; Toscano *et al*., 2014). Chia seeds contain high fiber and linolenic fatty acid which may reduce the creatinine level of the rabbits. *Salvia hispanica* controls blood pressure (BP) and its associated cardiometabolic factors. Also, Chia flour could reduce ambulatory and clinical BP in both treated and untreated hypertensive individuals (Toscano *et al*., 2014; Al-Othman *et al*., 2006).

For rabbits fed with un-oxidized sunflower oil, the ALT level was normal. Oxidized sunflower oil increased ALT level in rabbits. In the group which was fed with Chia seeds, ALT level was in normal range. Oxidized oil promotes serum ALT level significantly (Khan *et al*., 2017; Al-Othman *et al*., 2006; Zeb *et al*., 2019), leading to hepatotoxicity. Unoxidized oil is beneficial for liver as it has oleuropein that protects hepatocytes from damage. It had been observed that oxidative stress induced by rancid oils leads to liver injury, which caused an increase in the ALT level (Zeb *et al*., 2019; Aguilera *et al*., 2002). In

our study, unoxidized oil had no effect on cholesterol level, while oxidized sunflower oil elevated the cholesterol level in rabbits. Chia seeds had a positive effect on lowering blood cholesterol level. Similar to our results, blood cholesterol levels were increased by oxidized olive oil in rats (Khan *et al*., 2017; Kritchevsky *et al*., 2000) and by oxidized sunflower in rabbits (Khan *et al*., 2022). The Chia seed significantly decreased serum cholesterol level when compared to oxidized and un-oxidized sunflower oil treatments. Oxidized sunflower oil elevates the blood cholesterol level, while chia seed decreases its level. After absorption, it increases serum cholesterol level and may lead to atherosclerosis (Lou *et al*., 2012).

The oxidized sunflower oil fed group significantly increased the triglyceride level of rabbits, while Chia seed decreased the triglyceride level in rabbits. Thermally oxidized oils keep users at risk to arteriosclerosis and cardiovascular diseases due to the depletion of phenolic as well as antioxidants in its constituents. A significant ($p < 0.05$) increase in the triglyceride level was observed when rabbits were fed with the oxidized olive oil (Khan *et al*., 2017), oxidized sunflower oil (Zeb *et al*., 2017) and mustard oil (Carmena *et al*., 1996). The HDL concentration after feeding with oxidized sunflower oil was significantly decreased, whereas LDL values were increased as compared to normal group. Chia seed significantly increased HDL and decreased LDL level in rabbits. It has been suggested that HDL cholesterol and its constituents is increased after feeding sunflower oil, which helps in prevention of heart diseases (Lou *et al*., 2012; Carmena *et al*., 1996; Quiles *et al*., 1998). A significantly higher LDL susceptibility to oxidation was observed after sunflower oil intake in comparison with virgin olive oil, despite an increase in LDL α-tocopherol concentration in sunflower oil group (Aguilera *et al*., 2004). Histological studies revealed that un-oxidized sunflower oil caused no significant effect on the liver morphology and functions. Oxidized sunflower oil caused necrosis in centrilobular regions. It has also been reported that thermally oxidized ground nut oil leads to some liver diseases (Aguilera *et al*., 2004; Jimoh *et al*., 2004; Abdel Raouf *et al*., 2012). On the other hand, Chia seed caused no significant changes, as hepatocyte vacuolization and mild necrosis were present in the liver.

CONCLUSIONS

In comparison to unoxidized oil, it has been determined that oxidized sunflower oil significantly affects hematological and biochemical parameters of serum and alters liver histological pattern in rabbits. Chia seed on the other hand, minimizes harmful effects of oxidized oil and display antioxidant potential.

Declarations

Author contribution. Conceptualization, T.A and A.A.K.; Original draft, G.Y.Z, S.R and F.I ; Methodology, M.A and F.I.; Data curation: M.A and A.A.S.; Writing- review & editing, A.A.K, and T.A.; Visualization, T.A; Resources, M.A.; Project administration, T.A and A.A.K ; Funding acquisition, T.A.; Validation, F.A .; Investigation, S.R and A.A.K ; Formal analysis, T.A and S.R.; Supervision, T.A and A.A.K

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