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Original Research Article

The Protective Effect of *Echinops spinosus* Alcohol Extract on Toxicity Induced by The Administration of Repeated Deep-Fried Oil in Experimental Rats

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Abstract

The present work aimed to assess the impact of Echinops spinosus L. (ES) alcohol extract on the toxicity caused by the repeated administration of deep-frying oil (RDFO) in experimental rats. A phenolic bioactive compound of ES was quantified using HPLC. Group (I) rats (negative control) were fed a basal diet. Group (2) rats were fed a basal diet and gavaged 200 mg/kg b.w. of ES extract. Group (3) rats (positive control) were fed a basal diet and given (RDFO) at a dose of 5 g/kg b.w. Group (4) rats were fed a basal diet and given 200 mg/kg b.w. of ES extract+ (RDFO) at a dose of 5 g/kg for 35 days. Body weight gain (BWG), feed intake (FI), feed efficiency ratio (FER), and relative weight of the kidneys, liver, and heart were estimated. Analysis was conducted on serum biochemical parameters and antioxidant/oxidant markers. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), **γ**-glutamyl transferase, creatinine, urea, uric acid, lactate dehydrogenase (LDH), creatine Kinase-MB (CK-MB), total cholesterol, triglycerides, fasting blood glucose, malonaldehyde (MDA), and nitric oxide (NO) were elevated by the administration of (RDFO). The adverse effects caused by repeated exposure to deep oil were improved by administering ES alcohol extract to rats. These findings indicate that ES extract may have a beneficial effect on the toxicity caused by the administration of (RDFO). In conclusion, the administration of ES alcohol extract can enhance the effects of (RDFO) administration on rats.

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1. Introduction

Lipids are said to be the second major energy source, after carbohydrates. They are vital to absorb and transport lipid-soluble vitamins through the bloodstream [1]. Edible oil (e.g., soybean oil) is an excellent source of essential amino acids, antioxidants, and vitamins such as B, E, **β**-carotene, and tocopherols [2]. Vegetable oils such as soybean oil, olive oil, palm oil, and sunflower oil are a well-known dietary component reported by various researchers that prevents cell and tissue damage through antioxidant properties, atherosclerosis, cancer, Alzheimer's disease, and improve cardiovascular activity, and immune functions [3].

During the preparation of fried food products, the oil is burnt repeatedly at 150°C to 200°C in association with the exposure to air, moisture, and an unhealthy environment that leads to different chemical reactions, for example, oxidation, hydrolysis, polymerization [4]. This configuration change causes polyunsaturated fatty acids to acquire undesirable properties associated with saturated fatty acids, such as the correlation between increased serum cholesterol levels and higher lower-density lipoprotein (LDL) cholesterol [5]. Also, the oxidation of oil results in the

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formation of various products, some of which are toxic. For example, radical species generated during oxidation damage some biomolecules (membrane lipid, protein, and DNA), manifesting in cell dysfunction, aging, cardiovascular, and neurodegenerative disease [6]. A previous study has been also showing that deep-fried oil consumption causes the retardation of glycerolipid metabolism and alters microbiota structure in the gut [7].

It also reduces the antioxidant properties of the oils, leading to the production of free radicals, alcohols, aldehydes, ketones, hydrocarbons, cyclic, and epoxy compounds, as well as volatile or non-volatile components [8]. These oxidative products are both cytotoxic and genotoxic for human health, causing various injuries in the liver, kidneys, heart, circulatory system, and intestine [9]. Thus, harmful changes in fats and oils by thermal oxidation during deep-frying increase the peroxide value, free fatty acid contents, lipid peroxidation products, and formation of triacylglycerol polymers and dimmers [10].

Continuous consumption of oxidized oils contributes to liver, heart, and kidney dysfunction. The enzymes alanine transaminase, aspartate transaminase, lactate dehydrogenase, etc, are destroyed or denatured [11]. *In vivo* studies showed that hypercholesterolemia due to altered lipid metabolism can cause renal damage like glomerular necrosis in Wistar rats [10] .

For centuries, herbal medicines have been intended to prevent, treat, and cure diseases. The World Health Organization (WHO) announced that 80% of the world's population depended on traditional natural compounds for their medicinal health cases [12]. ES is one of the curative Worts of the *Asteraceae* family. Its roots and fruits have recently been used as an adjuvant in infections, diarrhea, hemorrhoids, labor pains, heart pain, migraine, and neuralgia. ES's chief components are alkaloids, polyacetylene thiophene, flavone glycoside, and benzothiophene glycosides besides echinopane, which were isolated from the roots, with verified antioxidant, anti-inflammatory, antiapoptotic, and neuro-modulatory effects [13]. ES is a curative plant with proven pharmacological and biological effects, including anti-inflammatory, antioxidant, and antibacterial competencies [14]. Traditional medicine frequently treats disorders associated with inflammation using it. The inside portion of the inflorescence is used in treating diabetes mellitus, postpartum care, and renal disorders. The fruits and roots of ES are used as a spice in Morocco and Cameroon, as an abortifacient in Algeria, and to relieve labor pains and neuralgia. Pregnant women are given a decoction of the roots in water or olive oil to stimulate uterine contractions [15]. Additionally, it is used as a diuretic or depurative for diabetes, to treat liver disorders, and for stomach pain, indigestion, and loss of appetite. These behaviors are closely related to the concentrations of secondary metabolites from various groups, including alkaloids, sesquiterpenes, flavonoids, and polyphenolic chemicals, in various ES parts [16].

Therefore, this study aimed to explore the ameliorative potential effects of ES extract on toxicity induced by the administration of (RDFO).

2. Materials and Methods

2.1. Materials

2.1.1 ES plant

ES plant powder was obtained from Agriculture Seeds, Herbs and Medicinal Plants Company, Cairo, Egypt. Corn oil and starch were purchased from the local market. Casein, cellulose, vitamins, minerals, dextrin, L-cysteine, and choline chloride were obtained from the Cairo Company for Chemical Trading, Cairo, Egypt. Raw (uncooked) vegetable oil samples (before and after heating and frying) were collected from three different local restaurants for the preparation of street foods. The fried vegetable oil was repeatedly fried at 150 °C to 200°C for 10 hours daily. The temperature of fried vegetable oil was also measured by a thermometer just after frying. After that fried vegetable oil was cooled and filtered through the Whatman filter paper and stored at 4°C temperature for further analysis. Twenty-four male albino rats (*Sprague Dawley strain)* were obtained from the Helwan Station for Experimental Animals, Helwan, Cairo, Egypt, weighing approximately 150± 10g.

2.1.2 Oils

Raw vegetable oil (RVO) and (RDFO) were obtained from three restaurants that use the same type of oil in Tanta (EL-Gharbia governorate, Egypt)at the end of the working day. The oil is a mixture of soybean oil and sunflower oil. (RDFO) was deep or very dark brown, dense with a rancid taste and odor. The three fried vegetable oil samples were mixed to form the fourth sample given to rats.

2.1.3 Rats

Twenty-four adult male albino rats *Sprague Dawley* strain weighting (150± 10g) were housed in well-aerated cages under hygienic conditions and were fed on a basal diet for one week for adaptation, the rats were kept in individual plastic cages under controlled environments, with a temperature of 22 °C and a 12-hour light/dark cycle at the Faculty of Home Economics, Al Azhar University, Egypt. Rats have unrestricted access to food and water. All experiments followed the National Institute of Health's Guiding Principles for Animal Care and Use.

2.2 Methods

2.2.1 Determination of peroxide value (PV) in oil samples

It measures peroxide content in an oil sample that quantifies iodine released from potassium iodide (KI). Oil samples (known weight) were firstly dissolved in acetic acid (CH_3COOH), then chloroform ($CHCl_3$) and a mixture of saturated KI were included in the solution. Then, the amount of free iodine was determined by titration with

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standard sodium thiosulphate ($Na_2S_2O_3$), where starch was used as an indicator [17] using the following equation:

$$PV = \frac{V \times N \times 1000}{Ws}$$

Sample weight (Ws), (N): normality of (Na2S2O3),(V):Volume of (Na2S2O3)

2.2.2 Determination of phenolic compounds

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatography equipped with an autosampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards [18].

2.2.3 Preparation of ES extract

The plant extract was prepared according to the method used by Benrahou et al. [19]; precisely 100 g of ES plant was extracted by macerated with ethanol (90%) for 48 hours stirring at room temperature. The extract was filtered and evaporated at 40 ° C using a rotary evaporator(R-100, Buchi, Japan). The dried extract (15g) was stored at 2-8°C until use.

2.2.4 Experimental design

Twenty four rats were divided randomly into 4 groups, each with six rats.

Group (I) rats were fed a basal diet as a negative control.

Group (2) rats were fed a basal diet and given 200 mg/kg bw of ES extract [20].

Group (3) rats were fed a basal diet and given (RDFO) at a dose of 5 g/kg bw for 35 days orally and served as a positive control group

Group (4) rats were fed a basal diet and given 200 mg/kg bw of ES+ (RDFO) at a dose of 5 g/kg bw for 35 days.

Body weight and feed intake were checked twice a week. Ultimately, animals were weighed and fasted overnight. Rats were then euthanized exsanguination.

2.2.5 Biological evaluation

At the end of the experiment, feed intake, body weight gain, relative organs weight, and feed efficiency ratio were calculated.

2.2.6 Biochemical analysis of serum

After the sacrifice of rats, blood samples were collected from the hepatic portal vein of each rat into dry clean centrifuge tubes. Serum was carefully separated by centrifugation of blood samples at 3500 rounds per minute (rpm) for 15 minutes at room temperature, transferred into dry clean Ebendorf tubes, and then frozen at - 20°C for later determinations. Serum samples were used to determine blood glucose, serum enzyme creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ - glutamyl transferase determination (GGT), albumin, and total protein. Globulin was calculated according to the following equation:

Globulin = Total protein – Albumin.

Serum total bilirubin, direct bilirubin, and indirect bilirubin was calculated according to the following equation:

Serum Indirect Bilirubin (g/dL) = Total Bilirubin (g/dL) - Direct Bilirubin (g/dL).

Serum uric acid, creatinine, urea nitrogen, total cholesterol, triglycerides, and HDL-C were determined. Serum VLDL-C and LDL-C were estimated. Also, Superoxide dismutase (SOD), glutathione peroxidase (GP_X), Catalase (CAT), nitric oxide (NO), Lipid peroxide (LPO) as malondialdehyde (MDA) were determined in serum.

2.2.7 Histopathological examination:

Livers, kidneys and hearts were taken and immersed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. They were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with hematoxylin and eosin for examining liver parts, according to Carleton, 1979 [21].

2.2.8 Statistical analysis:

One-way analysis of variance (ANOVA) was used, followed by the Duncan test, in SPSS software to calculate the difference between means at $P \leq 0.05$. The data was presented as a mean ± standard deviation (SD).

3. Results

Data presented in Table.1 shows the Peroxide value of RVO and (RDFO). The results revealed that the Peroxide value was a significant ($p < 0.05$) increase in (RDFO) in all samples compared with RVO.

Table 1: Peroxide value of raw vegetable oil and (RDFO)

Groups	(RVO) meq O_2/kg	(RDFO) meq O_2/kg
Sample 1	5.00±1.00^a	12.00±2.00^d
Sample2	4.57±1.16^a	18.33±1.52^c
Sample3	4.33±1.53^a	26.67±3.05^b
Sample4	6.33±1.53^a	40.00±2.00^a

Each value represents the mean of three replicates ±SD. Means with different letters in the same column differ significantly at p≤0.05

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Echinops spinosus was analyzed for its phenolic compounds. The results shown in Table 2 indicate that higher content of Chlorogenic (19270.95 µg/g), Ferulic (228.71µg/g) and Gentisic (168.63µg/g), While the lowest compounds are Chrysin (2.13µg/g) and Qurecetin (11.04µg/g).

Table 2: phenolic compounds in *Echinops spinosus* (µg/g)

Phenolic Compounds	Results (µg/g)
Gentisic	168.63
Cateachin	51.31
Chlorogenic	19270.95
Ferulic	228.71
Rutin	12.04
p-coumaric	54.49
Cinnamic	19.91
Qurecetin	11.04
Chrysin	2.13

Feed intake (FI), body weight gain % (BWG), and feed efficiency ratio (FER), shown in Table 3, have significantly increased in the positive control group compared to the standard group. Groups treated with ES extract showed significant decreases (P<0.05) in (FI and BWG %) when compared to the control group(+ve). Rats administrated ES showed non-significant decreases (p≤0.05) in FER compared to the positive control group. Rats administrated ES plus (RDFO) showed significant decreases compared to the positive control group.

Table 3: The protective effect of alcohol extract of ES on FI, BWG %, and FER in rats fed on (RDFO) (Mean±SD)

	FI (g /35 days)	BWG(%)	FER
Control –ve	690.83±1.80^{c}	21.63±1.53^{c}	0.065 ±.013^{b}
ES	682.82±2.69^{d}	21.53±.67^{c}	0.062±.005^{b}
Control +ve	744.83±1.43^{a}	30.29±1.58^{a}	0.089±.013^{a}
ES+ (RDFO)	716.33±2.25^{b}	27.36±3.76^{b}	0.085±.010^{a}

FI: food intake, BWG: Body weight gain, FER: Feed efficiency ratio, RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at p≤0.05 using one one-way ANOVA test, while those with similar letters are non-significant.

As shown in Table 4, relative kidney, liver, and heart weight increased in the positive control group compared with the negative control group. However, the ES-treated groups showed significant decreases (p≤0.05) when compared to the (+ve) control group.

The data in Table 5 reveals that the mean values of AST, ALT, and GGT in the control+ve group were significantly higher compared with the control –ve group. In contrast, all other treated groups were significantly decreased compared with the control+ve group at p≤0.05.

Table (4): The protective effect of alcohol extract of ES on relative organ weight (kidney, liver, heart) in rats fed on (RDFO) (Mean±SD)

	Relative Kidney weight (% BW)	Relative Liver weight (% BW)	Relative Heart weight (% BW)
Control –ve	0.58±0.04^{c}	3.14±0.35^{c}	0.29±0.005^{c}
ES	0.54±0.02^{d}	2.95±0.22^{d}	0.28±0.032^{d}
Control +ve	0.64±0.03^{a}	3.98±0.34^{a}	0.40±0.027^{a}
ES+ (RDFO)	0.61±0.01^{b}	3.65±0.12^{b}	0.35±0.008^{b}

BW: Body weight, RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at p≤0.05 using one-way ANOVA test, while those with similar letters are non-significant

Table (5): The protective effect of alcohol extract of ES on serum liver enzymes (ALT, AST, and GGT) in rats fed on (RDFO) (Mean±SD).

	AST (U/L)	ALT (U/L)	GGT (U/L)
Control –ve	95± 2.36^{c}	26.66±2.87^{c}	7.56±1.18^{c}
ES	72 ±3.22^{d}	17.66±2.87^{d}	6.36±.65^{d}
Control +ve	229.33±2.73^{a}	53.66±1.36^{a}	17.00±1.11^{a}
ES+ (RDFO)	140.66±4.13^{b}	41.66±2.06^{b}	12.86±1.30^{b}

aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ - glutamyl transferase determination (GGT),RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at p≤0.05 using one-way ANOVA test, while those with similar letters are non-significant.

The results in Table 6 demonstrate that the mean value of total protein, albumin, and globulin significantly decreased in the control+ve group compared to the standard rat group. The groups treated with ES extracts were significantly increased compared with the control+ve group at p≤0.05.

Table (6): The protective effect of alcohol extract of ES on total protein, albumin, and globulin in rats fed on (RDFO) (Mean±SD)

	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Control –ve	7.20± .26^{b}	4.20± .08^{b}	3.00± .17^{b}
ES	7.56±.18^{a}	4.40±.15^{a}	3.16± .13^{a}
Control +ve	5.62±.40^{d}	3.26±.22^{d}	2.36± .18^{d}
ES+ (RDFO)	6.26± .22^{c}	3.66±. 13^{c}	2.60±.08^{c}

RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at p≤0.05 using one one-way ANOVA test, while those with similar letters are non-significant.

According to the data in Table 7, the (+ve) control group's average values of total bilirubin, direct bilirubin, and indirect bilirubin were significantly (p≤0.05) higher than the (-ve) control. All handled groups recorded a significant decline in all parameters compared to the control (+ve).

The data in Table 8 reveal that the +ve control average lipid profile values (TC, TG, LDL, and VLDL) increased

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significantly compared to the - ve control group. All treatments significantly decreased in all parameters compared to the +ve control. On the other hand, HDL in the +ve control significantly reduced compared to that of normal rats. The groups treated ES extracts showed an increase compared with the control+ve group.

Table (7): The protective effect of alcohol extract of ES on serum liver enzymes (total bilirubin, direct bilirubin, and indirect bilirubin) in rats fed on (RDFO) (Mean±SD)

	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)
Control –ve	0.22±0 .01 [a]	0.06±0 .008 [b]	0.16±0.023 [c]
ES	.20±0.01[a]	0.02±0.005[a]	0.18± 0.008 [b]
Control +ve	0.46±0.02 [c]	0.18±0.02[d]	0.28±0 .010 [a]
ES+ (RDFO)	0.26±0.01[b]	0.11±0.01[c]	0.15±0.018 [b]

RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at p≤0.05 using one one-way ANOVA test, while those with similar letters are non-significant.

Table (8): The protective effect of alcohol extract of ES on Total cholesterol and Triglycerides in rats fed on (RDFO) (Mean±SD)

	TC (mg/dl)	TG (mg/dl)	LDL-c (mg/dl)	HDL-c (mg/dl)	VLDL-c (mg/dl)
Control –ve	83.86± 3.60[c]	89.33± 0.57 [c]	21.33± 4.16 [c]	44.66± 0.57 [b]	17.87± 0.00 [c]
ES	65.53± 4.04[d]	71.00± 5.56 [d]	4.00±2. 00[d]	47.33± 1.15[a]	14.20± 1.00 [d]
Control +ve	130.33 ±3.05[a]	145.00 ±6.55 [a]	69.00± 8.00[a]	32.33± 5.50[d]	29.00± 1.00[a]
ES+ (RDFO)	101.93 ±4.03[b]	113.00 ±.57[b]	41.33 ±1.52[b]	38.00± 6.55[c]	22.60± 0.00[b]

TC: Total cholesterol, TG: Triglycerides, RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at p≤0.05 using one one-way ANOVA test.

The results in Table 9 show that the + ve control group's average serum creatinine, urea, and uric acid levels were significantly higher than their counterparts in the – ve negative control. Groups treated with ES extracts reduced these parameters considerably compared to the + ve control.

Table (9): The protective effect of alcohol extract of ES on kidney function (Creatinine, Uric acid, and Urea) in rats fed on (RDFO) (Mean±SD)

	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control –ve	0.64±0.02 [c]	25.66 ±1.36 [c]	2.23±0.023 [c]
ES	0.56±0.03[d]	17.00 ±3.09 [d]	2.06±0.008 [d]
Control +ve	1.32±0.11 [a]	57.66 ±5.96 [a]	3.21±0.036[a]
ES+ (RDFO)	0.91 ±0.02 [b]	43.00±1.54 [b]	2.74 ±0.028[b]

RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at p≤0.05 using one one-way ANOVA test, while those with similar letters are non-significant.

The data in Table 10 indicated that the mean fasting blood glucose values in the positive control group were significantly higher than in the negative control group. In treatment groups, blood glucose significantly decreased (p≤0.05) compared to the positive control.

Table (10): The protective effect of alcohol extract of ES on blood Glucose in rats fed on (RDFO) (Mean±SD)

	Fasting blood glucose mg/dl
Control –ve	86.0±2.3 [c]
ES	74.6±3.3 [d]
Control +ve	126.6±4.03 [a]
ES+ (RDFO)	94.7±4.6 [b]

RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at p≤0.05 using one one-way ANOVA test, while those with similar letters are non-significant.

Table 11 displays the serum LDH and CK-MB levels that were increased in the unhealthy rats compared to the standard control. They were lower significantly in all treatments than the + ve control.

Table (11): The protective effect of alcohol extract of ES on Lactate dehydrogenase (LDH) and Creatine Kinase-MB (CK-MB) in rats fed on (RDFO) (Mean±SD)

	LDH (U/L)	CKMB (ng/ml)
Control –ve	880.00±1.78 [C]	0. 36 ± 0.03c
ES	819.00±1.78 [d]	0.32 ± 0.01c
Control +ve	1455.20±2.68 [a]	0.58 ± 0.03 a
ES+ (RDFO)	1089.3 ±1.86 [b]	0.47± 0.03b

RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at p≤0.05 using one one-way ANOVA test, while those with similar letters are non-significant.

Table 12 shows that compared to the negative control group, the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GP_X) considerably decreased in the positive control group, but they elevated in the therapy groups.

Table (12): The protective effect of alcohol extract of ES on serum antioxidant enzymes (CAT, SOD, and GPX)in rats fed on (RDFO) (Mean±SD)

	CAT (U/L)	SOD (U/ml)	GP_X (mU/ml)
Control –ve	1.89± 0.14 [b]	129.76±2.22[b]	92.10±1.54 [b]
ES	2.37±0.24 [a]	131.23±2.06 [a]	93.80±3.50 [a]
Control +ve	0.98±0.09 [d]	71.70±2.01 [d]	52.10±3.72 [d]
ES+ (RDFO)	1.42±0.09[c]	91.50±3.04 [c]	73.60± 2.62 [c]

RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at p≤0.05 using one one-way ANOVA test, while those with similar letters are non-significant.

The data in Table 13 show that the deep-fried oil group's mean MDA and NO values were significantly more significant than the standard group's. Compared to the

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positive control rats, every treated with ES extracts in the different remediations decreased significantly ($p \leq 0.05$).

Table (13): The protective effect of alcohol extract of ES on Lipid peroxidation parameter (MDA) and nitric oxide (NO) in rats fed on (RDFO) (Mean±SD)

	MDA (nM/ml)	NO (µM/L)
Control –ve	6.15±0.60 [c]	0.42± 0.054 [c]
ES	5.05±0.27 [d]	0.35±0.025 [d]
Control +ve	17.25±2.24 [a]	1.53±0.030 [a]
ES+ (RDFO)	9.50 ±0.65 [b]	1.15 ±0.005 [b]

RDFO: repeated deep-frying oil. Means with different letters in the same column differ significantly at $p \leq 0.05$ using one one-way ANOVA test, while those with similar letters are non-significant.

Histological Results

Figure 1: Microscopic pictures of H&E stained hepatic sections show normal hepatocytes, central veins, and opened sinusoids. Hepatic sections show few fat vacuoles in a few hepatocytes (thick black arrow) with opened sinusoids in group (1) Control –ve (A) and group (2) ES (B).Hepatic sections show marked hydropic degeneration in many hepatocytes (thick black arrow) with congested blood vessels (thin black arrow) and closed sinusoids in the group (3) Control +ve (C). Hepatic sections show marked hydropic degeneration in many hepatocytes (thick black arrow) in group(4) ES+ (RDFO) (D).

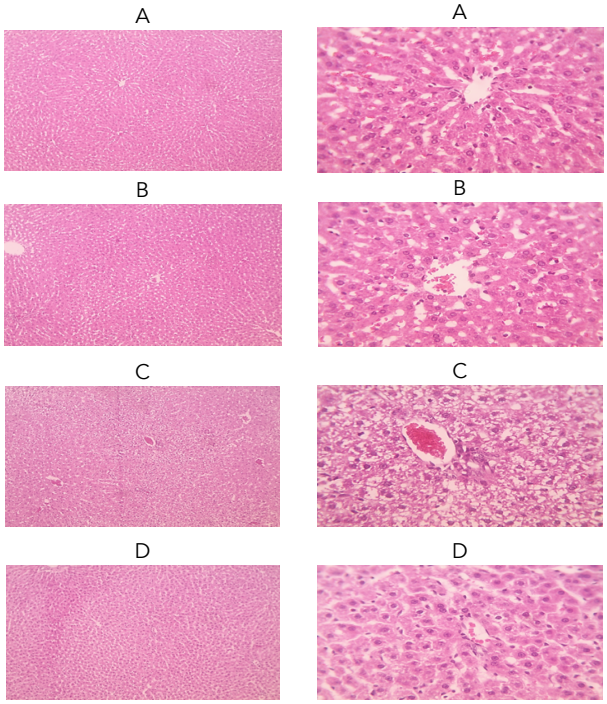


Fig. 1. Representative images of Haematoxylin and Eosin stained hepatic sections (x400) from (A) Normal Control group, (B) ES group, (C) Control +ve group, (D) ES+ (RDFO).

Figure 2: Microscopic pictures of H&E stained renal cortical sections show normal glomeruli& tubules in control –ve (A) and ES (B) groups. Renal cortical sections show marked ballooning degeneration in epithelial cells lining many tubules in control +ve (C) (thick black arrow). Renal cortical sections show mild ballooning degeneration in epithelial cells lining fewer tubules in (thick black arrow) in the group ES+ (RDFO) (D).

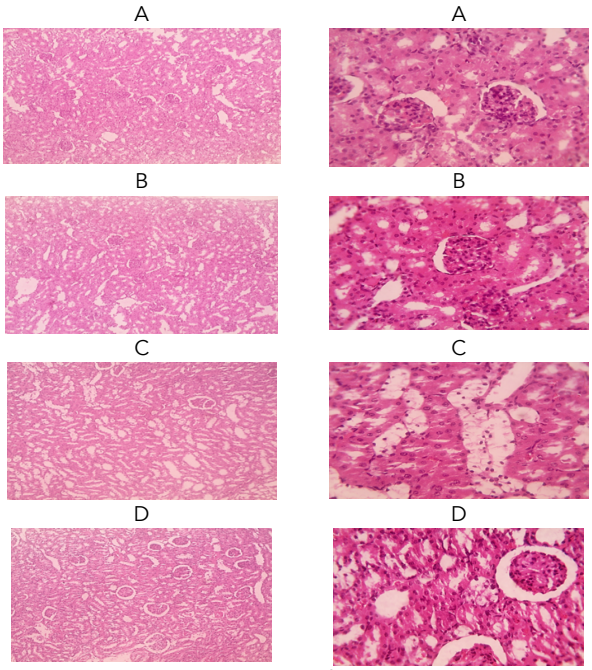
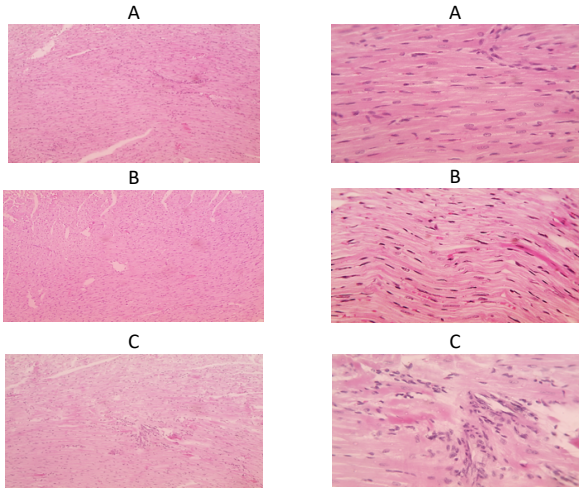


Fig. 2. Representative images of Haematoxylin and Eosin stained renal cortical sections (x400) from (A) Normal Control group, (B) *ES* group, (C) Control +ve group, (D) *Es* +(RDFO).

Figure 3: Microscopic pictures of H&E stained cardiac sections show normal muscle fibers with minimal interstitial tissue in the control –ve (A) and ES (B) groups. Cardiac sections show marked hyalinization (thick black arrow) and degeneration (arrowheads) in many muscle fibers with perivascular edema (thick black arrow), endothelial cells hyperplasia (curved arrow), and few leukocytic cells infiltration (thick white arrow) in the Control +ve (C) group. Cardiac sections show fat vacuoles in a few muscle fibers (arrowheads) in group (4) ES+ (RDFO) (D).



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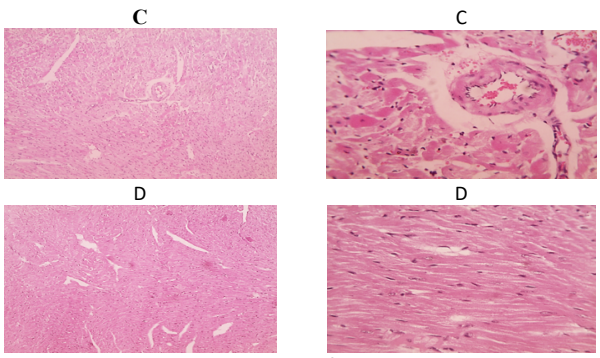

Fig. 3. Representative images of Haematoxylin and Eosin-stained cardiac sections (x400) from (A) Normal Control group, (B) ES group, (C) Control +ve group, (D) *ES*+ (RDFO).

4- Discussion

Plant oils become thermally oxidized after several rounds of heating to 150°C, generating oxygen-derived free radicals and hydroxylated products that are harmful to tissues [22]. Many wild plants are used in traditional medicine and pharmaceutical preparations. The beneficial effects of medicinal plants usually depend on their antioxidant bioactive phytochemical levels [23, 24]. Therefore, the current search and screening for beneficial plants with potential bioactive properties and the concomitant isolation and characterization of these bioactive compounds are necessary for fighting diseases [25]. Different plant parts of ES which can be used as traditional medicine and a source for some nutraceuticals contain antioxidant phytochemicals such as phenols, flavonoids, cardiac glycosides, glucosinolates, alkaloids, terpenes, steroids, coumarins which are characterized by free radical scavenging abilities, anti-inflammatory action, anticancer, anti-aging, and protective action for diseases [26].

The present results are in harmony with Xin-Fang et al. [27], who mentioned that heated palm olein increased the levels of peroxide, angiotensin-converting enzyme, and lipid peroxidation and reduced the level of heme oxygenase. Fresh palm olein and palm olein heated once had lesser effects on lipid peroxidation and were better than repeatedly heated palm olein. The more frequently the oil was reheated, the higher the peroxide index. These findings agree with Wazed et al. [28], who revealed that fresh oils have peroxide values lower than ten meq O_2/kg, and before oil becomes rancid, its peroxide value must be between 20 and 40 meq O2/kg. The peroxide value also increased with the temperature of the oil samples and their contact with air. The results are in harmony with Bustani & Soni [29], who mentioned that the amount of peroxide increased due to constant exposure to heat in all types of oil (reused oil). The high peroxide values mark the rise in lipid peroxidation products, mainly peroxide produced at the frying time.

The presented results agree with those of Hegazy et al. [20]. Joshi et al. [26] showed that ES could be used as traditional medicine and a source for some nutraceuticals containing antioxidant phytochemicals such as phenols, flavonoids, cardiac glycosides, glucosinolates, alkaloids, terpenes, steroids, and coumarins characterized by free radical scavenging abilities, anti-inflammatory action, anticancer action, antiaging action, and protective action for disease. Tungmunnithum et al. [30] and Al Masoudi and Hashim [31] found that total phenols and flavonoids are considered the most important bioactive compounds in plants due to their ability to enhance antioxidant and antimicrobial activity. Zitouni-Nourine et al. [32] reported that the phyto complex of the aerial parts from EShad shown the presence of 22 flavonoids, including Hespirtin, Hesperidin (, Luteolin-6-arabinose-8-glucose, Apigenin-6-arabinose-8-galactose and Apigenin-6-glucose-8-rhamnose, which were the primary metabolites.

Our results show that administration (RDFO) caused an increase in BWG and FI in rats. The results agree with Totani & Ojiri [33], who found that the reused deep-fried oil group tended toward higher diet ingestion than the negative control group. The difference in the amount of food ingested recovered oil seems acceptable for the experimental rats to fulfill their appetite. Also, results supported by Feleke et al. [34] cleared a significant increase in BWG. The rise in BWG might be due to the increase in the total triglyceride level of rats following deep-fried oil consumption. Khalifa et al. [35] showed that (FI) values of the +ve control group (fed 4% reheated sunflower oil.) increased compared with -ve control rats. The present results are in harmony with Ahmad et al. [36], who said that an increase in weight and nourishment was observed by using thermally oxidized coconut oil in mice due to the accumulation of unchanged metabolites in body tissues. The accumulation of these unchanged metabolites causes subcellular damage and thus affects cell metabolism, which increases body weight (obesity) and is a leading cause of several disorders. These findings are in line with Feleke et al. [34] and Dammak et al. [37], who reported that ES flower reduced body weight gain; this might indicate that the plant could be used as a weight loss agent for obese humans because lipid binding in the gastrointestinal tract reduces fat absorption.

Also, Our results show that the administration of reused deep-fried oil caused an increase in liver, kidney, and heart weight. These results are in line with those reported by Totani & Ojiri [33], and Thabet & Abou-Elkhair [38] found that growth of the liver and kidney volume after the use of degraded oils (repeatedly boiled oil) due to specific fractions of the heated fats, total polar compounds can result in growth retardation, increased liver and kidney weights, and disorders of the enzyme system. Also, Ambreen et al. [39] showed that following long-term oil treatment caused liver weight gain in repeatedly heated mixed vegetable oils fed animals, both in high and low doses, was statistically significant in comparison with the normal control group. These results are parallel with those of Senaphan et al. [40], who concluded that ferulic acid, found in Espinosa's as a phenolic compound, decreases

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liver weight. The reduced liver weight in FA-supplemented rats is probably due to the suppression of liver cells' proliferation, which reduces oxidative stress and insulin resistance, thereby normalizing lipid metabolism and suppressing inflammation, leading to an alleviation of the fatty liver. The results are in harmony with Yan et al. [41], who reported that chlorogenic acid (CGA) found in Espinosa's as a phenolic compound reduced liver weight in mice.

These results are in agreement with Chaturvedi et al. [42] and Ghobadi et al. [43] showed that the level of AST increased in rats consuming repeatedly heated oils. This increase could be due to oxidative stress-induced damage of liver cells following consumption of oxidized heated oil. The presented results are in agreement with Abarikwu et al. [44] and Abarikwu et al. [45] found that increased activities of GGT in the per oxidized coconut oil group may be due to hepatocellular necrosis, which causes an increase in the membrane damage of cells, resulting in the release of this enzyme in the blood circulation and suggests that the peroxide content in the oil exerts a pro-oxidant effect on the liver. Our results were supported by Jane et al. [11], who reported that the ALT and AST levels of the test group were higher than those of the control group. The increased level of enzymes suggests hepatic injury in the animals fed with reused oil because continuous consumption of oxidized oils contributes to liver dysfunction. Also, Amsalu et al. [46] found that the serum levels of liver damage biomarkers ALT and AST of experimental groups were increased significantly relative to the control groups because repeatedly and long-time heated oil causes damage to liver tissues as the liver is a major organ involved in energy metabolism. Hence, the effects of oxidized edible oils on the liver are tangible. The results agree with Srinivasan et al. [47], who indicated that ferulic acid (FA), which is found in Espinosa's as a phenolic compound, decreased lipid peroxidation, improved antioxidant status, and thereby prevented the damage to the liver and leakage of enzymes ALT, AST, and GGT. This is mainly because of the antioxidant-sparing action of FA. This finding is supported by Dammak et al. [37], who mentioned that ES methanolic extract significantly decreased plasma AST and ALT to near normal values, as compared with the infected mice because of the reported antioxidant effects of Espinosa's flower, which in turn lead to decreased free radical generation and reduced oxidative damage of the liver.

These results align with those reported by Falade et al. [6], who found that a significant decrease in the plasma and liver total protein and albumin of groups fed thermally oxidized palm oil diets compared to the oxidized palm oil diets altered protein synthesis in the liver due to cirrhosis and has been chronically damaged lead to albumin decreased. In 2024, Ahmad et al. [36] observed lower serum levels of total protein, globulin, and albumin due to frequent use of oxidized recycled coconut oil, leading to liver damage. The results agree with those of Fauzi et al. [48], who found that total protein, globulin, and albumin levels in the treated group with chlorogenic acid increased significantly. Chlorogenic acid has an anti-inflammatory effect that could reduce inflammation and has high antioxidants.

These results are in line with those reported by Falade et al. [6], who found that an increase in the total bilirubin of groups fed thermally oxidized palm oil diets due to the liver becomes irritated, alterations in membrane architecture of the cells of the liver, hence, an effect on the liver integrity. These findings are in line with Abarikwu et al. [45], who stated that there was also a significant increase in the serum profiles of bilirubin in rats fed per oxidized coconut oil, bilirubin levels may rise in the blood, suggestive of liver injury. Ahmad et al. [36] reported that higher levels of total bilirubin are due to frequent oxidized recycled coconut oil use. Wu et al. [49] found that the serum total bilirubin and direct bilirubin in chlorogenic acid-treated rats were decreased. Chlorogenic acid treatment improved them. It exerts an anti-inflammatory effect and reduces liver inflammation. Also, the results are supported by Zhu et al. [50], who noticed that levels of total bilirubin were improved and decreased after chlorogenic acid treatment. Finally, Dammak et al. [37] cleared that ES methanolic extract significantly decreased plasma bilirubin to near normal values due to their antioxidant activities. ES flower decreased free radical generation and liver oxidative damage.

Shastry et al. [51] indicated that an increase in total cholesterol (TC) and triglyceride (TG) levels in the animals treated with reused oils increase in triglyceride level after oil ingestion may be due to the increasing availability of substrate-free fatty acids for esterification and histopathological study supports the above findings suggesting the vital organs damage from toxic substances from the reused palm and sunflower oils. Rayhan et al. [52] confirmed that the TG level was significantly higher in the FVO group when using deep-fried vegetable oil rather than RVO repeatedly. Also, Feleke et al. [34] found elevated TC and increased total TG levels in rats due to following deep-fried oil recycled at least five times consumption. This finding is emphasized by Bustani & Soni [29], who reported that the thermally oxidized palm oil diets increased plasma total cholesterol. In 2022, Yan et al. [41] suggested that chlorogenic acid found in Espinosa's as a phenolic compound enhances hepatic lipolysis and reduces TG synthesis and fatty acid transportation in the liver.

In 2023, Yuan et al. [53] discovered that p-coumaric, a phenolic compound found in Espinosa's, can decrease TC and TG concentrations in serum and the liver in lipid-overloaded mice and HepG2 cells. This reduction in TC and TG was correlated with the promotion of liver lipase activity and the enhancement of protein expression in lipid metabolism.

Rayhan et al. [52] and Cui et al. [54] showed that serum low-density lipoprotein followed an increasing trend in deep-fried vegetable oil (FVO) fed groups while high-density

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lipoprotein showed an inverse effect. These results align with those reported by Jane et al. [11], who concluded that low-density lipoprotein (LDL) levels increased compared with normal control; high levels of LDL from consumption of vegetable oil used for frying increased. Also, VLDL level increased compared with normal control due to the generation of lipid peroxidation products formed due to exposure of the oil to heat for an extended period. Zeb & Khan [55] and Lounging et al. [56] suggested that the consumption of frying oils may negatively influence the lipid profile, leading to an increase in total and LDL cholesterol. This could be attributed to ingesting oxidized LDL through fried oil samples. The oxidation of these molecules generally leads to oxidized LDL, which is taken up by macrophages, and they accumulate to form foam cells. The accumulation of foam cells in the vascular subendothelium contributes to the development of atherosclerotic plaques and the onset of atherosclerosis. Also, Zeb & Khan [55] and Lounging et al. [56] mentioned that the increase in triglyceride concentration after ingestion of fried oil could be due to the presence of abundant free fatty acids in these oils and their availability as an esterification substrate in the formation of these molecules. The results agree with Totani & Ojiri [33], who found that rats fed on a deep-fried oil diet exhibited elevated levels of LDL-cholesterol in conjunction with decreased HDL-cholesterol because deep frying causes lipid oxidation polyunsaturated fatty acid-containing phospholipids in cell membranes and lipoproteins can be oxidized by toxic substances produced during deep frying. Wan et al. [57] indicated that chlorogenic acid exhibits cholesterol Lowering and Fatty Liver attenuating Properties by up-regulating the gene expression of PPAR-a in hypercholesterolemic rats induced with a cholesterol diet. Also, Senaphan et al. [40] and Jain & Surana [58] reported that ferulic acid (FA) decreased LDL and VLDL-C and increased HDL-C in serum. FA can inhibit cholesterol synthesis via competitive inhibition of HMG-CoA reductase. Thus, this antihyperlipidemic potential of FA may be due to its ability to inhibit HMG-CoA reductase activity. These findings align with Dammak et al. [37], who observed that treating hypercholesterolemic mice with Espinosa's flower methanolic extract induced a significant decrease in plasma LDL cholesterol concentrations compared to the hypercholesterolemic mice.

In contrast, Espinosa's flower extract improved the level of HDL-cholesterol because Espinosa's has high antihyperlipidemic activity in mice. This could be explained by the richness of the plant in polysaccharides and **β**-carotene, which is responsible for an increase in HDL-cholesterol, and that polysaccharides are firmly liable for the observed hypolipidemic effects due to the ability of the plant to hasten the decomposition of free radical species generated during cholesterol administration.

These results agree with Ould Amara-Leffad et al. [59] reported that oxidative oil (reused oil) has a nephrotoxic effect shown by the increase of urea, creatinine, and uric acid. The increase in urea and creatinine is explained by high protein catabolism and by the smaller reabsorption at the tubular; in other words, a change in glomerular filtration, which shows the settlement of renal insufficiency glomerular filtration change could be caused by angiotensin ll (it is pro-oxidation) hidden due to filtration and over. This finding is emphasized by Amsalu et al. [46], who found that the serum urea, creatinine, and uric acid levels increased in groups treated with fried oils. Repeatedly fried oils lose their ability to scavenge free radicals; oil loses its antioxidant ability as its duration and frequency of frying increase; antioxidants such as **α** tocopherol and **γ**-tocotrienol within the oil are degraded faster than those in other oils due to more oxidation of fatty acids, chronic feeding of oxidized oil induces organ damages and dysfunctions repeatedly and long-time heated oil causes the damage of tissues and changes like enzymes and their increase in the blood. These results by Rayhan et al. [52] showed that the concentration of creatinine and urea in the serum group of fried vegetable oil (FVO) reused several times in the rats was significantly increased compared to a group of raw vegetable oil (RVO) because frequent consumption of fried vegetable oils causes high blood fats which stimulate the rate of development of kidney disease and poor kidney function, so the glomeruli harden, leading to acute kidney disease, the glomerular filtration rate decreases and the concentration of creatinine, urea, and uric acid increases in the blood. These results are in agreement with several studies [20,60,61], which revealed that treatment with ES reduced serum urea, creatinine, and uric acid levels. ES extract contains squalene that displays an effective anti-inflammatory effect at low concentrations via stimulating the body's immune system and thereby competing with several ailments. Also, natural antioxidants play an important role in the prevention of nephrotoxicity since the methanol extract of ES revealed the highest polyphenolic content, phenol and flavonoid compounds, total tannin contents, and the most effective antioxidant potential. The findings of the present work are in agreement with Rizk et al. [14], who reported that a group of rats that were injected with the extract ES significantly decreased the level of urea, creatinine, and uric acid because Espinosa's has nephroprotective effect by improving antioxidant defense system and restraining inflammation and apoptosis.

Chao et al. [62] showed that the oxidized frying oil (OFO) diet resulted in glucose intolerance in both rats and mice due to hyperinsulinemia rather than hyperinsulinemia. The decreased circulating insulin levels may decrease glucose uptake by skeletal muscle and adipose tissue and impair glucose tolerance. The mechanism of hyperinsulinemia caused by high OFO consumption may involve impaired insulin secretion due to impaired function of pancreatic beta cells or increased liver extraction of insulin from the portal blood. It is known that OFO feeding induces higher

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oxidative stress, which may result in oxidative damage to the pancreas.

The oxidized oils, upon ingestion, increase the serum total cholesterol, LDL-cholesterol,glucose and ALT levels of the rats, while triacylglycerol and HDL-cholesterol decreased significantly. Similar observations were reported for the supplementation of thermally oxidized vanaspati ghee to the rabbits [13]. Our results are in agreement with a recent study, which also showed that thermally oxidized sunflower oil increases the serum total cholesterol and LDL-cholesterols while decreasing HDL-cholesterol [19]. It is therefore concluded that thermally oxidized oil, when fed to rabbits, rats, or other animals, produces similar results irrespective of the type of edible portions.

Also, Khan et al. [63] cleared that the oxidized oils, upon ingestion, increased the serum glucose levels of the rats in the positive control group. Zhuang et al. [64] found that intakes of peanut oil and refined blended plant oil used for deep-frying were strongly associated with higher type 2 diabetes (T2D) risk.

Tunnicliffe et al. [65] found that chlorogenic acid, which is found in Espinosa's as a phenolic compound, lowers the postprandial rise in blood glucose concentration because the chronic intake of chlorogenic acid lowers glucose absorption when consumed with or close to a meal and reduces the risk of type 2 diabetes due to by slowing the rate of glucose appearance from the intestine into the circulation. Also, Hasanuddin et al. [66] mentioned that chlorogenic acid decreases blood glucose because chlorogenic acid compounds can reduce intracellular hyperglycemia by regulating fat and glucose metabolism through AMP-activated protein kinase activation. Benrahou et al. [19] showed that ES extract reduced blood sugar significantly than the control group by this therapeutic effect. Its action has confirmed that some of it slows down the absorption of glucose by reversibly modulating the action of enzymes (**α**-amylase and **α**glucosidase) responsible for the breakdown of complex carbohydrates into monosaccharides.

Hamsi et al. [67] confirmed that LDH activity was increased in the heated virgin coconut oil group. It caused tissue damage due to inflammation caused by the reactive oxygen species that was generated by the thermal oxidation of repeatedly heating the oil, and this caused endothelial cell injury. These findings are in line with Rayhan et al. [52], who reported that the level of creatine kinase (CK-MB) has increased in rats fed fried oil. Also, Islam et al. [10] showed that the increased serum cholesterol level of fried mustard oil-fed rats was responsible for the development of atheromatous plaques in the coronary arteries, which led to myocardial infarction with an elevation of serum CK-MB. These results agree with Kheiry et al. [68], who found that pre-treatment with p-coumaric acid, which is found in Espinosa's as a phenolic compound, effectively suppressed LDH production in heart tissue of Lipopolysaccharide rats. It is an antioxidant and anti-inflammatory agent and can neutralize the effects of free radicals in heart tissue and prevent inflammation in the heart. Also, Liu et al. [69] demonstrated that augmented LDH and creatine phosphokinase activities and the increased infarct size elicited were significantly mitigated in the rats treated with ferulic acid for four weeks. Anjali et al. [70] confirmed that ferulic acid, which was found in Espinosa's as a phenolic compound, decreased serum levels of LDH and CK-MB rats. This suggests the effective cardio protection activity of it.

Falade et al. [6] found that oxidized palm oil, compared to fresh palm oil, increased plasma and liver MDA in rats. Also, Ambreen et al. [39] and Venkata & Subramanyam [71] found that significant reduction in SOD, GPx, and lower CAT levels in a high dose- repeated heated mix vegetable oil (H-RHMVO) and low dose- repeated heated mix vegetable oil (L-RHMVO) groups in comparison with normal control while the MDA levels in H-RHMVO and L-RHMVO groups increased very significantly in comparison with normal control group because antioxidant enzyme activity is inhibited by the toxic intermediates formed due to high-fat diet consumption and accumulated H2O2 and oxygen-radicals also support the rapid formation of hydroxyl radicals. Abarikwu et al. [45] found that a significant increase in hepatic and renal MDA in per oxidized coconut oil due to oxidized dietary oils enhances in vivo lipid peroxidation, which is accompanied by elevated levels of lipid peroxidation products such as MDA in tissues, per oxidized coconut oil also decreased CAT activity.

Our results are supported by Ghasemi-Sadabadi et al. [72], who reported decreased SOD, GPx, and CAT with oxidized oil supplementation in the diet. Further, the inclusion of 4% oxidized oil in diets quadratically increased MDA concentration in blood. Ahmad et al. [36], Gopinath et al. [73], and Abdelnour et al. [74] showed that feeding of deep--oxidized oil reduced the levels of SOD. ES treatment significantly mitigated the overproduction of TNF-␣.

Bagdas et al. [75] stated that chlorogenic acid significantly decreased the MDA and NO levels and increased the SOD levels in rats. It has been reported to exhibit potent antioxidant and anti-inflammatory. In 2019, Hegazy et al. [20] confirmed that ES treatment significantly mitigated the overproduction of NO. Abou Zaid et al. [76] found that cinnamic acid demonstrated a magnificent recovery in MDA level because cinnamic acid had a curative effect that inhibited the peroxidation of cell membrane lipids and kept its integrity and was able to ameliorate enzymatic and non-enzymatic antioxidant defense system and to prevent the lipid peroxidation in these tissues. Dammak et al. [37] noticed that ES flower was found to increase the SOD and GPx activity of the antioxidant enzymes. Finally, Rizk et al. [14] reported that ES extract supplementation was able to influence redox change as seen by the suppression of MDA and NO production as compared with the non-pretreated by lowered MDA, and NO levels and improved activities of GPx, CAT, and SOD.

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Shastry et al. [51] suggested that toxic substances from the reused palm and sunflower oils damaged the vital organs. The liver cells were swollen, and chronic inflammatory cell infiltration and microgranules were observed near the portal area. The heart showed a congested myocardium and little vacuolation in the papillary muscle. The kidney showed tubular cells in the medullary region, as well as cytoplasmic and nuclear vacuoles. Occasional tubules contained eosinophilic materials in the lumen. Rayhan et al. [52] showed that some degenerative changes like enlarged central vein with rapture necrosis of liver parenchymal cells, spot necrosis of liver parenchymal cells, leukocyte infiltration, and accumulation dilation of blood sinusoid were found in both FVO fed groups and RVO groups) but the injuries. In the photomicrograph of the kidneys of Wistar rats, the structure of the renal corpuscle collecting tubule, distal convoluted tubule, and proximal convoluted tubule were severely altered in both FVO and RVO groups. However, it was massive in the FVO group compared to the RVO-fed group. Moderate levels of myocardial necrosis accumulation of inflammatory cells and vacuolization and enlargement of the myocardium were observed in the FVO + RVO group rats, and these effects were more severe in the FVO group. The results agree with Ahmad et al. [36], who found that histology of the liver and heart in the group of oxidized coconut oil mice showed that papillary muscles had few vacuolization and a congested myocardial state. Hegazy et al. [20] noticed that histological studies of the ES group rat showed normal histological structure of renal tubules and glomeruli in kidney tissue. These results are in agreement with Rizk et al. [14], who reported that normal tissue architecture of the renal cortex was visible upon microscopic examination of stained renal slices from the control and ES extract. However, renal sections from rats given high and low dosages of ES extract demonstrated some amelioration of renal histological abnormalities.

5- Conclusion

In the present study, it is clear that (RDFO) became rancid and contributed to high peroxidation. Consumption of oxidized vegetable oil alters the level of lipid profile as well as liver, heart, and kidney function biomarkers in the circulatory system .also, The results of this study cleared the favorable protective action of ethanolic extract of ES against(RDFO-induced oxidative damage through its anti-oxidative and anti-inflammatory effects, which was confirmed biochemically and histopathologically. Therefore, ES can serve as an effective therapeutic agent with a low incidence of side effects and can be used as an inexpensive alternative that can be consumed in the daily diet to confer protection against (RDFO).

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