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Regular paper

Roundup-induced biochemical and histopathological changes in the liver and kidney of rats: the ameliorative effects of *Linum usitatissimum* oil

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The present study was undertaken to evaluate the protective effects of *Linum usitatissimum* oil (LuO) against sub-chronic Roundup (RDP)-induced toxicity and oxidative stress in rats. Rats were divided into four groups: control group (no treatment), RDP group (Roundup at 269.9 mg/kg b.w.), LuO group (0.5 g/kg b.w. of LuO) and RDP+LuO group (RDP and LuO simultaneously). LuO decreased the ferric reducing antioxidant power (FRAP) (IC_{50}=10.36 µg/ml) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (IC_{50}=22.85 mg/ml) in the tested tissues. The 30-day exposure of rats to RDP caused an increase in serum hepatic and renal markers: AST, ALT, ALP, LDH, γGT, bilirubin, urea, and creatinine. In addition, SOD, CAT and GST activities decreased by 43%, 61%, and 61%, respectively, while GPx activity, MDA and PCOs levels increased by 80%, 46%, 25%, respectively. LuO treatment alleviated hepatotoxicity in RDP-treated rats, showing improved levels of oxidative stress biomarkers and plasma biochemical parameters. The histological examination of the liver and kidney confirmed the changes in Roundup-treated rats and demonstrated the protective role of LuO.

Key words: roundup, linseed oil, oxidative stress, hepatotoxicity, nephrotoxicity, rats

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Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; LDH, lactate dehydrogenase; γ-GT, gamma glutamyl-transpeptidase; GSH, reduced glutathione; GPx, glutathione peroxidase; LPO, lipid peroxidation; MDA, malondialdehyde; TBA, thiobarbituric acid; TBARS, TBA reactive substances; NO, nitric oxide; AOPP, advanced oxidation protein product; PCO, protein carbonyl; RDP, Roundup; SOD, superoxide dismutase; CAT, catalase; GST, glutathione-S-transferase; DNPH, carbonyl group with 2,4-dinitrophenylhydrazine.

INTRODUCTION

Organophosphate (OP) pesticides are among the most widely used synthetic chemicals to control a wide variety of pests and insects in agriculture, homes, gardens, veterinary practice and industry (Dar *et al.,* 2019). Roundup (RDP) is an organophosphorus compound glyphosate (N-(phosphonomethyl)glycine), an active ingredient of many herbicides, such as Roundup® TURBO, and the most commonly used herbicide active ingredient in the world (El-Shenawy 2009). Glyphosate and its primary degradation product aminomethylphosphonic acid (AMPA) are detected in soils and sediment, ditches, drains, rainwater, rivers, and streams (Rebai *et al.,* 2017). Roundup is marketed as a non-selective, broad spectrum and post-emergence herbicide due to its high water solubility. It kills plants by inhibiting the synthesis of aromatic amino acids needed for protein formation (Battaglin *et al.,* 2014). Roundup is slightly toxic to fish, practically non-toxic to amphibians and aquatic invertebrate animals, and exhibits low oral and dermal acute toxicity to humans (Larsen *et al.*, 2012). Over the last two decades, toxicological research has shown the induction of oxidative stress after exposure to pesticides as a possible mechanism of toxicity. According to several studies, the pesticides, including roundup, cause overproduction of reactive oxygen species (ROS) in intra- and extracellular spaces, resulting in the disruption of pro-oxidant equilibrium/cellular oxidative balance and leading to the oxidative stress state (El-Shenawy, 2009; Heritier *et al.,* 2017). In humans, glyphosate is not fully absorbed after oral administration (approximately 20% of the dose administered is excreted in the urine after 48 h), being mainly eliminated unchanged via the feces. The absorbed glyphosate is poorly metabolized, gets widely distributed in the body, does not undergo enterohepatic circulation and is rapidly eliminated without bioaccumulation ("Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate", 2015).

Indeed, in a study conducted in 2011 and 2012 in Denmark (Knudsen *et al.*, 2017), urine spot samples of mothers (n=13) and children (n=14) revealed concentrations of glyphosate above the limit of detection (LOD, 2.5 µg/L) in both urban and rural dwelling populations. It was found that when ROS formation exceeded the scavenging ability of antioxidant defenses, free radicals accumulated and increased oxidative damage to critical biomolecules such as enzymes, proteins, membrane lipids and DNA (Mohammadirad & Abdollahi, 2011). Several studies indicate that glyphosate and GLP-based herbicide (GBH) intoxication causes oxidative stress by generating free radicals and induces tissue lipid peroxidation. In response to oxidative damage, organisms have developed enzymatic antioxidant defense systems, which are represented mainly by superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase. These enzymes detoxify ROS and maintain a healthy redox state in a highly synchronized

and cooperative manner (Tang *et al.,* 2017; Yazdinezhad *et al.,* 2017). Organophosphate pesticides was found to repress cytochrome P450 and two other enzymes (glutathione-S-transferase and G-6-P dehydrogenase) essential for xenobiotics detoxication in the body (Almeida *et al.*, 2017). However, RDP toxicity towards serum acetylcholinesterase (AChE), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and acid phosphatase (AP) has been recently determined (Rebai *et al.,* 2017; Tang *et al.,* 2017). RDP poisoning can affect several organs, including the brain, heart, kidney, liver and reproductive system (Dai *et al.,* 2016; Tizhe et *al.,* 2014; Turkmen *et al.,* 2019). Several epidemiological studies have shown the importance of increasing the antioxidant capacity of the body with exogenous antioxidants such as vitamins, carotenoids, trace elements and minerals (Djeffal *et al.,* 2015; Barkat *et al.,* 2015). Flaxseed or linseed (*Linum usitatissimum*) is an annual plant that belongs to the family Linaceae (Varghese *et al.,* 2017). The research study of Hendawi and others (Hendawi *et al.,* 2016) has shown that linseed oil obtained by the company El-capten contains high levels of unsaturated fatty acid: C18:1 (Oleic) (45.63%), C18:3 (γ-Linolenic) (8.93%) and C18:2 (Linoleic) (4.19%), as evaluated with gas liquid chromatography (GLC) of fatty acid methyl esters. In addition, it has been reported that linseed oil is among the most well-known edible oils in the world and is in demand as a nutritional supplement (Ye *et al.*, 2019; Zhang *et al.*, 2008). Being a source of α-Linolenic acid, it is a precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Rubilar *et al.,* 2010) and plays an active role in the cell membrane integrity (Karaca & Eraslan, 2013). Linseed is rich in n-3 polyunsaturated fatty acids (PUFA), especially omega-3 fatty acid that has pharmacological and therapeutic effects, in particular, antidiabetic, antioxidant, anti-inflammatory, anti-cancer and immunomodulatory. Linseed is also used for the treatment of cardiovascular diseases, dermatological, neurological and hormonal disorders, and metabolic syndromes such as obesity (Hendawi *et al.,* 2016; Jamilian *et al.,* 2017; Varghese *et al.,* 2017). *L. usitatissimum* is a source of healthy oil that has good anti-free radical properties and good oxidation stability, probably due to the changes in its fatty acid profile (Jamilian *et al.,* 2017). To the best of our knowledge, no study has examined the protective effects of LuO on RDP-induced hepatotoxicity and nephrotoxicity. Therefore, the present study was conducted to assess some biochemical parameters, antioxidant status and histopathological alterations in the liver and kidney of RDP-exposed rats. It also tried to determine whether the treatment with LuO could alleviate RDP-induced toxicity.

MATERIALS AND METHODS

Chemicals. The herbicide used in this study was a commercial formulation (Roundup® TURBO) containing the active ingredient glyphosate 450 g/l. The chemical name for glyphosate is N-phosphonomethyl glycine ($C_3H_8NO_5P$) (Fig. 1), with a CAS registry number 1071-83-6 and homologation number 05 43 087. This product is made by MONSANTO firm and distributed by Agro Consulting International, Algeria.

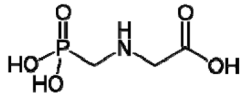

Figure 1. Chemical structure of glyphosate

Linseed Oil Resource. *Linum usitatissimum* oil was obtained from a local commercial market El-Captain (El-Obour City, Cairo, Egypt), a company for Extracting Natural Oils, Plants and Cosmetics. All the other reagents used in this study were purchased from Sigma Chemical Co. (St. Louis, MO).

Total phenolic acids content. Total phenolic acids content was measured using Folin–Ciocalteu reagent, following Wolfe and others (Wolfe *et al.,* 2003). The total phenolic acids content of plant fragments was expressed in mg of gallic acid equivalents per gram of plant dry matter (mg GAE/g DM) through the calibration curve with gallic acid.

Total flavonoids content. Total flavonoid content was measured using a colorimetric assay according to the method of Pourmorad and others (Pourmorad *et al.,* 2006). The concentrations of flavonoids, calculated based on a standard curve, were expressed in mg quercetin equivalent to (QE)/g dry matter (DM).

Total tannins content. Total tannins content was estimated according to the protocol developed by Julkunen-Tiitto (Julkunen-Tiitto, 1985). The results are expressed in mg of catechin equivalent (CE)/g DM.

DPPH radical scavenging activity. The antioxidant activity of LuO was measured in context of radical scavenging ability using the DPPH method of Blois (Blois, 1958). The antiradical activity was expressed as IC_{50} (mg/ml). The percent inhibition of DPPH free radical was calculated as follows:

$$I\ \% = \left(\frac{A\ blank - A\ sample}{A\ blank}\right) \times 100$$

Ferric reducing antioxidant power (FRAP) activity. The reducing power of a sample was determined using the method of Pan and others (Pan *et al.*, 2008), where absorbance of a sample was measured at 700 nm. An increase in absorbance of the reaction mixture indicated increased reduction of Fe^{+3}.

Animals and experimental design. All the protocols used in this study were approved by the Ethical Committee of Directorate General for Scientific Research and Technological Development at Algerian Ministry of Higher Education and Scientific Research, permit no PNR/SF 08/2012. Twenty-four male albino Wistar rats weighing 250±13 g (aged 8-9 weeks) were provided by Pasteur Institute (Algiers, Algeria). They were housed in cages at room temperature of 23±2°C and kept under standard conditions of an average relative humidity of 40% and a natural photoperiod. The rats were fed a standard laboratory diet (standard food, supplied by the "ONAB of Bejaia", Algeria) and water was available *ad libitum*. The animals were habituated to these conditions for one month prior the experiments. The rats were randomly divided into four groups of six animals each:

Control: rats had free access to water and commercial standard pelleted food;

RDP (RDP-Treated group): received RDP in their drinking water (269.9 mg/kg/B.W);

LuO (LuO-Treated group): rats were force-fed with LuO (0.5 g/kg B.W.);

RDP+ LuO (RDP+LuO-Treated group): rats received both RDP and LuO.

The dose of RDP used in this study represented 1/18 of LD_{50} (269.9 mg/kg B.W.). This dose was used in pre-

vious investigations as it is toxic, but not lethal to rats (El-Shenawy, 2009). The dose of LuO (0.5 g/kg B.W.) gave a good protection against Roundup toxicity (Hendawi *et al.,* 2016). During the treatment period (30 days), food intake and water consumption, as well as body weight of the animals, were monitored regularly. At the end of the experiment, the animals fasted overnight and their total body weight was recorded. They were then sacrificed by cervical decapitation without anesthesia in order to minimize the animals' stress.

Biochemical analysis. Rats' blood was collected into non-heparinized tubes and centrifuged for 15 min at 3000 rpm at 4°C. Serum samples were stored at –20°C for subsequent analysis of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), total bilirubin, total proteins, albumin, cholesterol, triglycerides, HDL, LDL, creatinine, urea and uric acid. All parameters were estimated using commercial kits from COBAS INTEGRA400 (glucose, ref. 2172682; AST, ref. 2056097; ALT, ref. 2056119; ALP, ref. 20766739 and 20759961; LDH, ref. 2055503; GGT, ref. 20759996; total bilirubin, Ref. 20737488; total proteins, ref. 2053039; albumin, ref. 20737461; Cholesterol, Ref. 20763012; Triglycerides, Ref. 2144620; HDL, Ref. 03038637; LDL, Ref. 03038866; creatinine, Ref. 20764345; urea, Ref. 2055660; uric acid, Ref. 2054728).

Tissue preparation. After the animals were sacrificed, their livers and kidneys were immediately removed, washed in a 0.9% NaCl solution and weighed. The relative weight of these organs (%) was calculated as g/100 g body weight. Then, one lobe of liver and another of kidney were used for histological examination. The remaining lobes of liver and kidney were homogenized on ice in Tris-Buffered Saline (1:2 *w/v*; 1 g of tissue with 2 ml TBS: 50 mM TRIS, 150 mM NaCl, pH 7.4) and the cell suspension was centrifuged (9000 rpm, 15 min at 4°C). The supernatants were divided into aliquots and then stored at –20°C for oxidative parameters analysis.

Histopathological examination. Samples of tissues (livers and kidneys) were fixed in 10% formaldehyde. They were dehydrated in a series of increasing alcohol concentrations (70–100%) (LEICA TP1020) and embedded in paraffin. The tissues were cut in 5 μm thick slices using microtome (LEICA RM2235), then deparaffinized and stained with hematoxylin and eosin (H&E) and examined using LEICA DM 1000LED microscope, following the method of Hould (Hould, 1984).

Measurement of lipid peroxidation levels. Malondialdehyde (MDA), which is the end product of oxidation of polyunsaturated fatty acids, was assessed as a marker for lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) as TBARS to produce a red colored complex that has a peak absorbance at 532 nm (Buege & Aust, 1978). The amount of TBRAS in a sample was calculated using an extinction coefficient of 1.56×10^5M/cm and expressed in nmol MDA/mg of protein.

Measurement of advanced oxidation protein product contents. The advanced oxidation protein product (AOPP) is a marker of oxidant-mediated protein damage. Its level was determined according to the method of Kayali and others (Kayali *et al.,* 2006) by the formation of dityrosine-containing and cross-linking protein products. The absorbance of the reaction mixture was immediately recorded at 340 nm. The concentration of AOPP was expressed in nmol/mg of protein.

Content of reduced glutathione. Reduced glutathione (GSH) content of liver and kidney homogenates was determined using a colorimetric technique described by Ellman (Ellman, 1959) and modified by Jollow and others (Jollow *et al.,* 1974), which is based on measuring optical absorbance at 412 nm that reflects the reduction of DTNB [5,5'-dithiobis-(2-nitrobenzoic acid)] by -SH groups of glutathione. The total GSH content is expressed in nmol GSH/mg protein.

Content of vitamin C. Vitamin C (ascorbic acid) level measurement was performed as described by Jacques-Silva and others (Jacques-Silva *et al.,* 2001), using the oxidation of vitamin C with copper to form dehydroascorbic acid. This product was further treated with 2,4dinitrophenylhydrazine to form an osazone, which, when treated with sulphuric acid, resulted in a colored product. Absorbance of the sample was measured at 540 nm and the result was expressed in mmol of ascorbic acid/mg of protein.

Measurement of antioxidant enzyme activities. The glutathione peroxidase (GPx) (E.C.1.11.1.9) activity in a sample was measured using the method of Flohé and Günzler (Flohé & Günzler, 1984), based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH). The absorbance of the sample was recorded at 420 nm. Specific GPx activity was expressed in mmol GSH/mg protein.

The glutathione-S-transferase (GST) (E.C.2.5.1.18) activity in the tissues was measured spectrophotometrically using the method of Habig *et al.,* (1974). It is based on the conjugated reaction of glutathione and 1-chloro-2,4-dinitrobenzene (CDNB) which, in turn, results in a new molecule that absorbs light at 340 nm. The GST activity is expressed in μmol CDNB/GSH /min/mg protein.

The catalase (CAT) (E.C.1.11.1.6) activity was measured according to Aebi (Aebi, 1984). This assay is based on the ability of the enzyme to degrade hydrogen peroxide followed by the decrease in absorbance at 240 nm using a UV/visible light measured for 1 min. CAT activity is expressed in μmol H_2O_2 consumed/min/mg protein.

The superoxide dismutase (SOD) (E.C.1.15.11) activity in a sample was determined with the method of Beyer Jr and Fridovich (Beyer Jr & Fridovich, 1987), using nitrotetrazolium blue tetrazolium (NBT) test and photo-reduction of riboflavin/methionine, which generates superoxide anion. In aerobic conditions, the mixture of riboflavin, methionine and NBT is blue. The presence of SOD inhibits the oxidation of NBT and changes the absorbance at 560 nm. The changes in samples absorbance were recorded after 20 min of incubation of the reagents and tissue samples. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%. The activity was expressed as units/mg of protein.

Content of protein carbonyl groups. Protein carbonyl levels in the samples were estimated with the method described by Levine and others (Levine *et al.,* 1990). The homogenates were incubated with 2,4-dinitrophenylhydrazine (DNPH) in 6M guanidine hydrochloride. As DNPH binds selectively to the protein carbonyl groups the absorbance at 370 nm changes. The level of protein carbonyls was expressed in nmol/mg of protein.

Protein content estimation. The protein content of tissue samples was measured spectrophotometrically at 595 nm using the method of Bradford (Bradford, 1976), with bovine serum albumin as a standard.

Statistical analysis. All data were expressed as means ± standard error means (S.E.M.) for six rats in each

Table1. Amounts of total phenols content, total flavonoids, condensed tannins and anti-radicular DPPH scavenging activity in LuO

Studied parameters	Values
Total phenolic acids content (mg GAE/100 g of LuO)	0.11±0.01
Total flavonoid content (mg QE/g LuO	0.32±0.01
Condensed tannins (mg CE/g LuO)	0.25±0.02
50% scavenging concentration (mg/ml) on DPPH radical	22.85±2.6
FRAP test (EC50 mg/ml)	10.36±1.59

n=3, GAE, gallique acide equivalents; QE, quercetin equivalents; CE, catechin equivalents.

group. The statistical analysis (comparison between all the groups) was performed with one-way ANOVA followed by Student's *t*-test to evaluate the significance of differences. $P<0.05$ was considered statistically significant.

RESULTS

Antioxidant activity of LuO

The total phenolic content of LuO product was 0.11 mg of CAE per 100 g of extract as shown in Table 1. The total flavonoid content is expressed as 0.32 mg of quercetin equivalent per gram of LuO. In addition, LuO contained 0.25 mg of condensed tannins, expressed as mg of catechin equivalent per gram. The antioxidant activity (DPPH) IC_{50} was 22.85 mg/ml, and ferric reducing antioxidant power (FRAP) was IC_{50}=10.36 µg/ml, showing the reductive potential of LuO.

Effects of treatment on general rat health

During the experimental period no deaths were observed in any group. The rats in the control and LuO groups showed no signs of systemic toxicity. However, the rats in RDP group showed clinical signs such as anorexia, loss of body weight, exhaustion, and inflammation of the salivary glands (the cause of drooling). All these clinical signs were related to the acute toxicity of RDP. Furthermore, the rats in RDP+LuO group showed some symptoms of anorexia and loss of body weight.

Effect of treatment on body, liver and kidney weight, food intake and water consumption

As shown in Table 2, the final body weight and the absolute liver and kidney weight of rats decreased significantly in the RDP, LuO and RDP+LuO groups of (–38%, –13% and –25%), (–21%, –14% and –22%), (–21%, –14% and –22%) and (–14%, –13% and –13%), respectively, compared to the control group. This decrease was associated with the reduction in food intake and water consumption in RDP, LuO and RDP+LuO groups (–19%, –14% and –33%), (–24%, –9% and –37%, respectively), when compared to the control group. However, the administration of LuO correlated with a significant increase in absolute liver and kidney weight when compared to rats from the RDP group.

Effect of treatment on serum biochemical parameters

As shown in Table 3, administration of RDP caused hepatic toxicity in rats. Serum glucose levels in rats from the RDP group tended to be higher than in control. Furthermore, the enzymatic activity of AST, ALT, ALP, LDH, total bilirubin and GGT was significantly higher in rats exposed to RDP than in the control group (48%, 37%, and 43%, 58%, 29% and 115%, respectively). In addition, urea and creatinine levels increased significantly in RDP group compared to the control group (11% and 16%), indicating nephrotoxicity. The levels of total protein and albumin in the serum were significantly lower in the RDP group. Similarly, the serum lipid profile in the RDP group showed a significant decrease in total cholesterol and triglycerides as compared to the control group (8% and 21%). However, the co-administration of LuO alleviated the RDP effect on blood biochemical variables.

Antioxidant defense status in the liver and kidney

Table 4 and Table 5 summarize the effects of RDP exposure and LuO administration on antioxidant defense systems in rat liver and kidneys. An increase in MDA (46%, 17% respectively), AOPP (18%, 14% respectively), and PCOs (25%, 15% respectively) was observed in the RDP and LuO-RDP groups compared to the control. On the

Table 2. Effects of treatments on body weight (g), absolute (g) and relative liver and kidneys weights (g/100 g bw), daily food intake (g) and drinking water consumption (ml) in experimental groups.

Studied parameters	Control	RDP	LuO	RDP+LuO
Initial Body weights (g)	249.6±2.7	250.33±4.64	250.5±7.29	249.66±7.86
Final Body weights (g)	299.5±11.4	235.5±4** p=0.0001	257.5±11.94	233.5±8.68 p=0.33
Absolute liver weights (g)	9.00±0.43	5.55±0.23*** p=0.001	7.79±0.21	6.70±0.49$^{\#\#}$ p=0.004
Absolute kidney weights (g)	1.85±0.09	1.59±0.03** p=0.013	1.61±0.07	1.59±0.05 p=0.48
Food intake (g/day/rat)	21.66± 0.69	17.43±1.18*** p=0.0002	18.69±0.66	14.51±1.23$^{\#\#\#}$ p=0.0001
Quantities of drinking water consumption (ml/day/rat)	32.65± 2.34	24.84±2.85*** p=0.00002	29.52 ±2.44	20.31± 2.02 $^{\#\#\#}$ p=0.0003
Quantities of RDP ingested (mg/day/rat)	–	67.75 ±7.79	–	55.39±5.52$^{\#\#}$ p=0.002

Values are given as mean ±SEM for groups of 6 animals each. Significant difference: LuO, RDP groups compared to the control ($^{**}p<0.01$, $^{***}p<0.001$), RDP+LuO compared to the RDP group ($^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$).

Table 3. Effects of treatments on serum biochemical parameters

*P*arameters studied	Control	RD*P*	LUO	RD*P*+LUO
Glucose	0.99±0.01	1.09±0.03** *p*=0.01	0.96±0.02	1.00±0.03# *p*=0.04
ASAT	221.06±24.12	330.42±20.06** *p*=0.004	272.42±6.21	266.3±11.73# *p*=0.05
ALAT	66.5 ±1.75	91.5±7.20** *p*=0.003	72.66±1.14	66.5±2## 0.003
LDH	2188±223.16	3472.6±205.67*** *p*=0.0008	2406±124.01	2085.5±142.06### *p*=0.001
AL*P*	104.13±8.53	149.13±13.61** *p*=0.009	106.4±3.02	114.51±3.18## *p*=0.01
GGT	8.01±0.55	17.3±1.38*** *p*=0.00001	7.96 ±1.2	8.33±1.24### *p*=0.0003
Bil.T	1.03±0.07	1.33±0.13* *p*=0.04	0.66±0.05	0.43±0.04### *p*=0.00002
*P*rot.T	83.85±1.11	75.8±2.25** *p*=0.004	85.56±1.93	83.86±1.51## *p*=0.006
Alb	33.42±2.27	26.98±1.62* *p*=0.02	35.85±1	33.58±2.04## *p*=0.01
Cholesterol	1.5±0.05	1.62±0.04* *p*=0.05	1.49±0.05	1.34±0.04### *p*=0.0004
Triglycerides	0.57±0.02	0.69±0.04* *p*=0.02	0.58±0.01	0.58±0.02# *p*=0.02
HDL	1.42±0.06	0.85±0.07*** *p*=0.0001	1.2±0.08	1.03±0.04# *p*=0.04
LDL	0.14±0.005	0.18±0.01** *p*=0.01	0.10 ±0.009	0.16±0.02 *p*=0.22
Urea	12.35±0.47	13.72±0.45* *p*=0.03	11.64±0.23	12.45±0.55## *p*=0.003
Creatinine	34.66±0.71	40.5±1.38*** *p*=0.001	32.33±1.31	38.66±1.02## *p*=0.15

Values are given as mean ±S.E.M. for groups of 6 animals each. Significant difference: LuO, RDP groups compared to the control (*p<0.05,**p<0.01,***p<0.001), #RDP+LuO compared to the RDP group (#p<0.05, ##p<0.01, ###p<0.001)

Table 4. Antioxidant defense status in liver of treated and control rats.

Studied *p*arameters	Control	RDP	LuO	RDP+LuO
MDA (nmol/mg protein)	23.41±3.13	34.33±1.71** *p*=0.005	16.13±1.35	23.43±1.34### 0.0002
AOPP (nmol/mg protein)	0.039±0.002	0.046±0.002** *p*=0.01	0.038±0.001	0.04±0.002# *p*=0.02
PCO (nmol/mg prot.)	80.46±3.68	100.53±2.8*** *p*=0.0007	81.16±2.25	82.83 ±4.75## *p*=0.004
GSH (µmol/mg prot.)	2.6±0.14	1.85±0.16** *p*=0.003	2.39±0.12	3.11±0.14### *p*=0.00003
Vitamin C (mmol ascorbic acid/mg of protein)	1.0±0.01	0.94±0.02* *p*=0.03	1.0±0.01	1.0±0.006# *p*=0.03
CAT (µmol H2O2/min/mg protein)	347.5±35.67	134.83±8.46*** *p*=0.00002	328.83±23	347.66±21.41### *p*=0.00003
GPx (nmol GSH/min/mg protein)	1.05±0.13	1.91±0.18*** *p*=0.001	0.95±0.12	1.32±0.12## *p*=0.01
GST (nmol/mg protein)	0.13±0.02	0.05±0.002*** *p*=0.001	0.1±0.007	0.08±0.01# *p*=0.006
SOD (U/mg de protein)	294.16±15.54	166.83±19.74*** *p*=0.0002	293.33±20.38	273.66±17.71### *p*=0.001

Values are given as mean ±S.E.M. for group*p*s of 6 animals each. Significant difference: LuO, RDP group*p*s com*p*ared to the control (*p<0.05,**p<0.01,***p<0.001), #RDP+LuO com*p*ared to the RDP grou*p* (#p< 0.05, ##p<0.01, ###p<0.001).

Table 5. Antioxidant defense status in kidney of treated and control rats.

Studied parameters	Control	RD*P*	LuO	RD*P*+LuO
MDA (nmol/mg protein)	28.07±1.18	32.92±1.31** *p*=0.01	18.89±1.92***	20.76±2.72### *p*=0.001
AOPP (nmol/mg of protein)	0.021±0.001	0.024±0.0007* *p*=0.05	0.023±0.002	0.02±0.002# *p*=0.08
PCO (nmol/mg prot.)	74.83±3.66	86.16±4.14* *p*=0.03	73.33±3.58	74.66±3.77# *p*=0.03
GSH (µmol/mg prot.)	0.75±0.03	0.63±0.03** *p*=0.01	0.72±0.03	0.68±0.01 *p*=0.14
Vitamin C	0.43±0.010	0.029±0.014* *p*=0.05	0.038 ±0.015	0.035±0.018 *p*=0.36
CAT (µmol H2O2/min/mg protein)	605± 8.72	418.5±52.02** *p*=0.002	571.83 ±68.04	471.01±35 *p*=0.21
GPx (nmol of GSH/min/mg protein)	0.57±0.07	0.76±0.06* *p*=0.05	0.56±0.04	0.45±0.05## *p*=0.002
GST (nmol/mg protein)	0.06±0.004	0.051±0.002* *p*=0.05	0.066±0.006	0.054±0.006 *P*=0.36
SOD (U/mg de protein)	162.16±13.03	73.83±7.3*** *p*=0.00002	197.83±21.45	134.5±7.31### *p*=0.00004

Values are given as mean ±S.E.M. for group*s* of 6 animals each. Significant difference: LuO, RDP group*s* compared to the control (**p*<0.05,***p*<0.01,****p*<0.001),#RDP+LuO com*p*ared to the RD*P* group (#*p*<0.05, ##*p*<0.01###*p*<0.001).

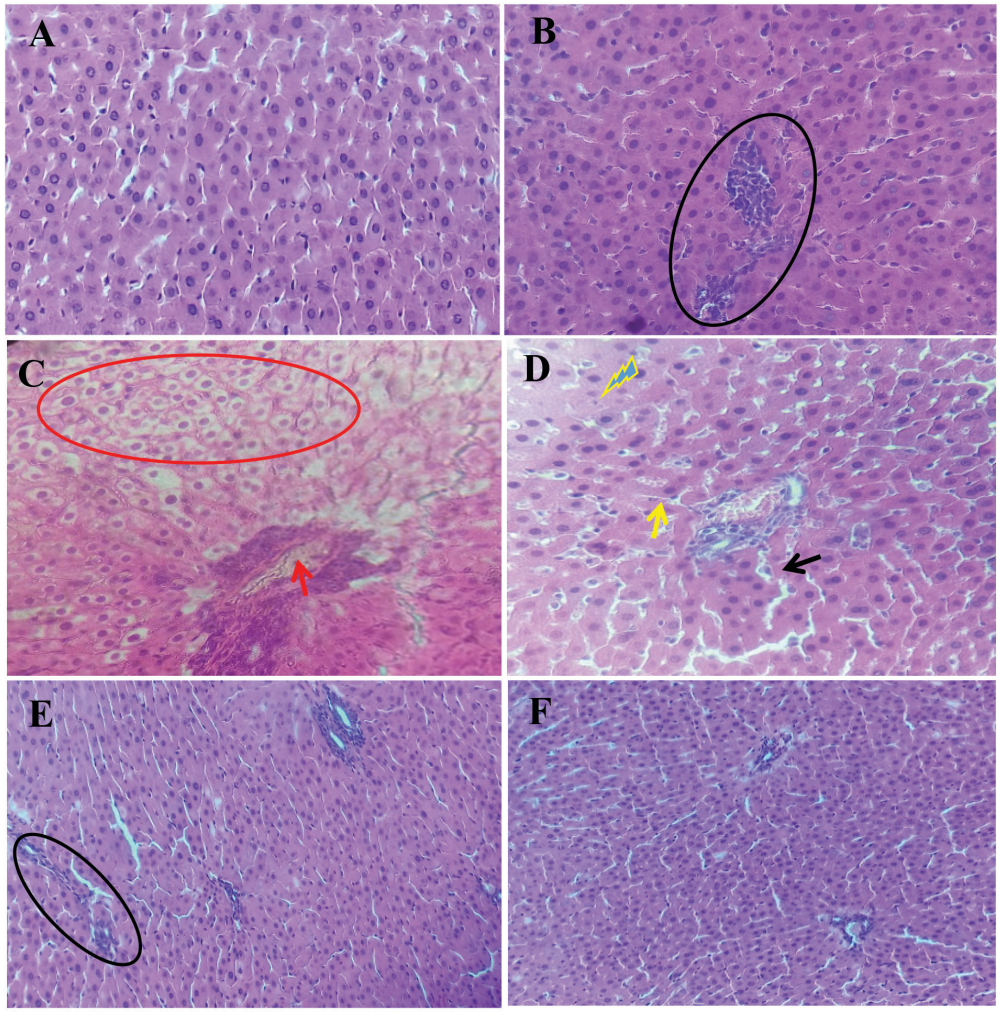

Figure 2. Effect of Roundup (RDP) and Linssed oil co-administrated with RDP on histopathological damages in the liver.
Controls (A, x40), treated with RDP (B to D, x40), LuO co-administrated with RDP (E, x40) and LuO (F, x40) after 4 weeks of treatment, as revealed by photomicrograph of H&E. Vascular congestion (red arrow), mononuclear infiltration (black circle), dilation of sinusoids (black arrow), and hemorrhage (yellow arrow), necrosis ( ) and degeneration and ballooning of hepatocyte (red circle).

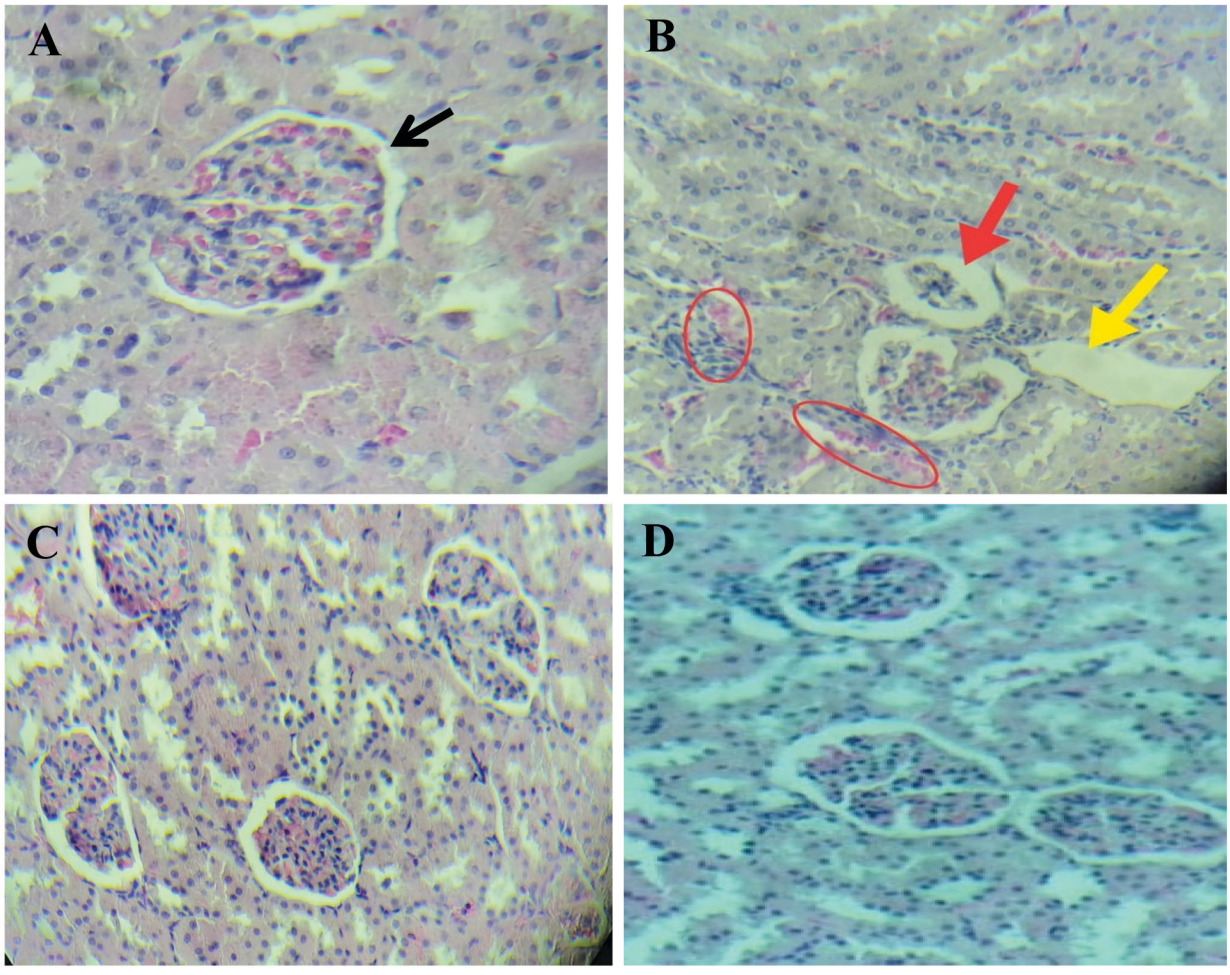

Figure 3. Effect of Roundup (RDP) and Linssed oil co-administrated with RDP on histopathological damages in the liver.
Controls (A, x40), treated with RDP (B, x40), LuO co-administrated with RDP (C, x40) and LuO (D, x40) after 4 weeks of treatment, as revealed by photomicrograph of H&E. Space of Bowman (black arrow), tubular dilatation (yellow arrow), hemorrhagic (circle) and atrophied and collapsed malpighian (red arrow).

other hand, the activity of enzymatic and non-enzymatic antioxidants significantly decreased in the RDP and LuO-RDP groups as compared to the control: GSH (–28%, –15% respectively), Vit C (–6%, –14% respectively), CAT (–61%, –30% respectively), GST (–60%, –14% respectively) and SOD (–43%, –54% respectively), and the level of GPx increased (80%, 31% respectively). In general, administration of LuO significantly improved the antioxidant parameters in the studied organs of rats treated with RDP.

Histopathological Results

Histopathological changes in rat liver and kidney resulting from RDP treatment are shown in Figs 2 and 3, Tables 6 and 7, respectively. The control rats presented normal hepatic morphology with distinct hepatocytes with prominent nuclei and no tissue damage (Fig. 2A). In contrast, liver sections of Roundup-treated rats showed severe structural damage characterized by mononuclear cell infiltration, periportal expansion, ballooning and degeneration of hepatocytes, necrosis, vascular congestion and sinusoidal dilatation (Fig. 2B to D). However, the livers of the rats from the LuO and RDP treated group had healthier histology than the rats from RDP-only treated group (Fig. 2E). No histological alteration in the liver was observed between the LuO and control groups (Fig. 2F). The histopathological kidney examination showed normal histology in the control group (Fig. 3A). However, the kidneys of rats exposed to Roundup exhibited glomerular dam-

Table 6. Semi quantitative scoring of histopathological changes in the liver sections of control and rats treated with Roundup (RDP), linseed oil (LuO) or their combination (RDP+LuO) after 4- week treatment.

Studied parameters	Control	RDP	Luo	RDP+LuO
Mononuclear cell infiltration	–	+++	–	+
Hepatic hemorrhage	–	+	–	–
Necrosis	–	++	–	–
Vascular congestion	–	+++	–	–
Ballooning and degeneration of hepatocyte	–	+++	–	–

(–) indicates normal, (+) indicates mild, (++) indicates moderate, and (+++) indicates severe.

Table 7. Semi quantitative scoring of histopathological changes in the kidney sections of control and rats treated with Roundup (RDP), linseed oil (LuO) or their combination (RDP+LuO) after 4- week treatment.

Studied parameters	Control	RDP	Luo	RDP+LuO
Atrophied Malpighian	–	++	–	+
Tubular dilatation	–	+++	–	–
Hemorrhagic	–	++	–	–

(–) indicates normal, (+) indicates mild, (++) indicates moderate, and (+++) indicates severe.

age characterized by reduced Bowman space, atrophied and collapsed Malpighian glomeruli, tubulo-interstitial involvement of hemorrhagic suffusion zones, and dilation of proximal convoluted tubules (Fig. 3B). However, the co-administration of LuO with RDP resulted in a reduction in Bowman's space and normal cell morphology in the kidney when compared to Roundup-treated group (Fig. 3C). No histological alteration in the kidneys was observed between the LuO and control group of rats (Fig. 3D).

DISCUSSION

Exposure to toxic substances such as pesticides in the environment can contribute to the emergence of oxidative stress in biological systems and metabolic imbalance (Djeffal *et al*., 2015). Many studies have been conducted to relate Roundup® use to liver damage and renal failure in animals (Tang *et al*., 2017) and a number of human diseases (Seneff & Orlando, 2018). Natural antioxidants have been considered to play an important role in counteracting the oxidative stress induced by various xenobiotics in tissues (Bouasla *et al.,* 2014). In our experiments, the phytochemical analysis of flaxseed oil revealed the presence of phenolic acids compounds, flavonoids and condensed tannins, with a noticeable antioxidant activity. The antioxidant activity of LuO has been evaluated on the basis of radical scavenging effect on the free radical DPPH, which has been widely accepted as a tool for estimating the activities of antioxidants on free radicals (Beroual *et al.,* 2017).

The obtained results demonstrated that exposure to Roundup® TURBO at 269.9 mg RDP/kg/day b.w. in drinking water for 30 days caused a significant decrease in the rats' body weight and their liver and kidneys weight. These results were consistent with previous studies in adult rats exposed to various pyrethroid insecticides widely used in agriculture: imidacloprid, deltamethrin and methomyl (Kapoor *et al*., 2011; Saoudi *et al*., 2011; Djeffal *et al*., 2015). We supposed that the decrease in body weight observed in RDP-treated rats was probably due to the lower food intake, as RDP exposure may account for a reduced water and food intake. Our results are in agreement with those published by Tang and others (Tang *et al.,* 2017) who have stated that the exposure to Roundup evokes a significant reduction in body weight, in liver and kidneys weight. Moreover, most physiological functions were modified in correlation with significant changes in food and water consumption. These observations were similar to those of the Çağlar and Kolankaya (Çağlar & Kolankaya, 2008) and Barkat and others (Barkat *et al.,* 2015). RDP and LuO treated rats also showed a significant reduction in body weight compared to control rats.

Flax is low in carbohydrates but extremely high in both soluble and insoluble fiber (Zhang *et al.,* 2008). In traditional medicine, linseed oil is used in the diet because taking flaxseed oil gives a feeling of fullness that lasts for a long time. As a result, the co-administration of LuO to RDP-treated group prevents the decrease in rats' absolute weight of liver and kidneys.

In the present study, a significant increase in blood glucose levels was recorded in Roundup-treated rats. This hyperglycemia may be due to an increase in insulin production and lesions in the islets of Langerhans and acinar cells caused by oxidative stress damage (Tizhe *et al.,* 2018). Many authors report that the elevation of glucose rate in organophosphorus may indicate the disruption of carbohydrate metabolism emanating from the increased degradation of hepatic glycogen (Al-Attar, 2015). On the other hand, the RDP-treated rats exhibited a significant increase in TG, serum cholesterol, and LDL-C compared to the control rats. Hypertriglyceridemia and hypercholesterolemia can be explained by an increase in lipid rate peroxidation and free radicals release. MDA tested in the liver and kidneys in RDP-treated rats suggested a high lipid peroxidation. HDL-C could protect LDL-C against oxidation in vivo, since the lipids of HDL-C are preferentially oxidized before those of LDL-C (Bowry *et al.,* 1992). Our results agree well with those obtained by (Al-Attar, 2015; Meligi & Hassan, 2017).

Flaxseed oil is rich in alpha-linolenic acid (ALA). It was shown that ALA supplementation improves glucose tolerance and reduces insulin resistance (Goncalves *et al*., 2018). The obtained results indicate that LuO administered orally attenuates the extensive changes in the energetic profile parameters such as glucose and lipid profile in RDP–treated rats (Costa *et al.,* 2018; Pilar *et al.,* 2017).

The liver is the first organ that protects the body from xenobiotics. Leak of liver enzymes such as ALT, AST, and ALP into the blood serum is a sign of hepatocellular damage and hepatic dysfunction (Bischoff *et al.,* 2018). In our results, a significant increase in the activity of ALT, AST, ALP, LDH, and GGT occurred in the serum of RDP-treated rats as compared to the control ones. This elevation is probably due to a disruption of enzyme metabolism due to increased membrane permeability and leakage of these enzymes from hepatocytes into the blood (Dar *et al.,* 2019). The present results were in line with the previous reports, which demonstrated that an increase in hepatic parameters was induced in rats exposed to Roundup and deltamethrin (Dar *et al*., 2019; Saoudi *et al*., 2011). Nevertheless, Çağlar and Kolankaya (Çağlar & Kolankaya, 2008) observed a decrease in transaminase activity in rats that received glyphosate-based herbicide during 5 to 13 weeks. This discrepancy is probably due to the body adaptation during long exposure to the chemical. A significant increase in bilirubin blood levels was also observed in the Roundup -treated rats compared to the control group, confirming liver damage and its histopathological changes. Our data are in line with the observation that pesticides exposure exhibits clear-cut abnormalities in the biomarkers of tissue damage (Kanbur *et al.,* 2015; Khan *et al.,* 2013). In our study, long-lasting exposure to RDP caused a significant

decrease in total protein and albumin levels in serum. These results are consistent with the findings of many other studies (Barkat *et al.,* 2015; Uchendu *et al.,* 2017), in which total protein and albumin levels in rats treated with organophosphorus pesticides were decreased. Irreversible changes induced by ROS, such as the formation of carbonylated proteins and the accumulation of these products in tissues, are usually associated with a permanent loss of protein function and are considered as indicators of oxidative damage and protein dysfunction (Fagan *et al.,* 1999). It is interesting to note that the co-administration of RDP and LuO partially suppressed the hepatotoxic effect of RDP. The reduction in activity of the key hepatic enzymes indicates the regeneration process of hepatocyte membranes. An increase in blood bilirubin and normalization of both albumin and total proteins was also noticed. Similar results demonstrated that LuO attenuated severe changes in hepatic biochemical parameters in rats treated with pesticides (Chavan *et al.,* 2013; Hana & Sae, 2013; Hendawi *et al.,* 2016). Another organ vulnerable to pesticide damage is the kidney, due to larger perfusion and increased concentration of excreted compounds that occur in renal tubular cells (Messarah *et al*., 2013). Serum levels of creatinine and urea were used as indicators of renal function. Elevated blood urea is known to be linked with an increased protein catabolism as a result of increased synthesis of arginase enzyme involved in urea production (Saoudi *et al*., 2011). In this study, increased serum creatinine and urea levels in rats treated with RDP reflected renal failure. Our results are similar to those obtained by Dedeke and others (Dedeke *et al.,* 2018) who monitored changes in the kidney biomarkers in rats exposed to RDP. Uric acid is a final product of purine degradation and is eliminated in the urine. In our study, the increase in serum uric acid may be explained by a strong degradation of nucleic acids (DNA and RNA). Therefore, the high level of circulating uric acid may be an indicator of the effectiveness of cellular defense against the deleterious effects of free radicals. This may be the cause of the increase of uric acid in Roundup -treated rats (El-Shenawy, 2009). LuO administrated to rats was found to inhibit the RDP poisoning effects on the renal dysfunction markers (Hana & Saed, 2013). Indeed, the imbalance between antioxidants and pro-oxidants causes damage to the integrity of the cell membrane (Slama *et al.,* 2018). The impact of ROS on biological membranes is analyzed using a test determining the degree of lipid peroxidation and protein oxidation. The increase in ROS level in the cell may be due to either overproduction of these reactive substances, or a decrease in the cell's capacity to neutralize them (Kirschvink *et al.,* 2008). Our research work demonstrated a significant increase in the level of MDA and AOPP in the liver and kidney in the RDP-treated group compared to the control group. The aromatic amino acids with a thiol group are the most sensitive to the oxidation and are a particular ROS target in proteins (Aydin *et al.,* 2010). Moreover, our results showed an increase in PCO, whose elevation may be due to the formation of adducts between certain amino acid residues and lipid peroxidation products such as MDA (Stadtman & Levine, 2006). These results are in correlation with previous reports (Abarikwu *et al.,* 2015; Hamdaoui *et al.,* 2016; Tang *et al.,* 2017; Yazdinezhad *et al.,* 2017), which suggested that organophosphate (glyphosate and chlorpyrifos) metabolism might generate reactive oxygen species (ROS), which in turn could lead to enhanced lipid peroxidation and protein oxidation. However, LuO supplementation causes a significant decrease in oxidative damage of protein and lipid peroxidation content in RDP/LuO rats. Accordingly, in our study, the LuO protection against RDP could be based on the presence of the above-mentioned antioxidants. The linseed oil could transfer phenolic hydrogen to a peroxyl free radical of a peroxidized n-3 polyunsaturated fatty acid (PUFA) (Yang, 2012). Similar findings are reported by Karaca and Eraslan (Karaca & Eraslan, 2013) and Pilar and others (Pilar *et al.,* 2017). In fact , the body is able to produce endogenous antioxidants to reduce the damage caused by ROS, including GSH, Vit C and certain enzymes, such as SOD, CAT, GST and GPx (Khaldi *et al.,* 2018). This thiol is abundant in living organisms in its reduced form (GSH). The reaction of glutathione with free radicals generates superoxide anion and glutathione in its oxidized form (GSSG). This could explain the fact that the cell attempts to maintain a low level of GSSG under physiological conditions and that some organs such as the liver and kidneys eliminate GSSG under oxidative stress (Cossu *et al.,* 1997). Vit C plays a role of a protector of the cell compartment against free radicals (Djeffal *et al.,* 2015). RDP-treated rats exhibited a significantly lower level of this antioxidant in the renal and hepatic tissues compared to the control rats. Our results revealed that the RDP administered in rats' drinking water caused a decrease in glutathione and vitamin C levels in the liver and kidneys. This was due to the oxidation of GSH to GSSG to protect cells against oxidative damage (El-Shenawy, 2009; Hamdaoui *et al.,* 2016; Kasmi *et al.,* 2018).

The treatment with LuO was confirmed to increase the level of GSH and vit C. This result may be due to the protection mechanism of linseed oil against Roundup -induced toxicity by the reduction of GSSH and stabilized GSH level. The GPx and GST antioxidant enzymes found in the cytoplasm play a major role in the conversion of H_2O_2 and organic hydro-peroxides into water and alcohol using GSH as a cofactor (Slama *et al.,* 2018). The significantly lower levels of GST in hepatic and kidney tissues in Roundup-treated rats may be a consequence of the decreased GSH level that is used to inhibit the enzymes and induce their inactivation in the case of excessive production of H_2O_2 due to lipid peroxidation (Bhondave *et al.,* 2014). In fact, the results of our experiment also showed a highly significant increase in GPx of rats after exposure to RDP. However, (Djeffal *et al.,* 2015) have described an increase in GPx activity, suggesting that elevated enzymatic activity counteracts the oxidative stress induced by methomyl, and affirming that it is a way of adaptation of the body. The activities of GPx and GST enzymes are in concordance with the study of (Abarikwu *et al.,* 2015). CAT and SOD antioxidant enzymes are the first line of defense against the detrimental effects of free radical damage. They also convert superoxide radicals into H_2O_2. This function is only effective if it is followed by the actions of CAT, since the H_2O_2 produced by SOD is subsequently cleaned by the CAT (Zemmouri *et al.,* 2017). This explains why in this study we found a decrease in enzymatic activity of SOD and CAT in the treated rats compared to the control group, in all the studied tissue compartments (Abdel-Daim *et al.,* 2019; Turkmen *et al.,* 2019). Rats co-treated with LuO showed a significant improvement of the antioxidant enzymes GPx, GST, CAT and SOD activity when compared to RDP-treated rats. These findings constitute further evidence of LuO powerful antioxidant potential (Hendawi *et al.,* 2016; Pilar *et al.,* 2017; Xu *et al.,* 2017; Wei Yang *et al.,* 2012).

Histopathological studies corroborated the biochemical analysis. In particular, Roundup -exposed rats showed the infiltration of inflammatory cells, presence of degenerating and ballooning hepatocytes, necrosis, glomerular damage, and dilation of proximal convoluted tubules impairment in rats' hepatic and kidney cells. These results are in agreement with those obtained in other studies (Abarikwu *et al.,* 2015; Al-Attar 2015; Hamdaoui *et al.,* 2016; Larsen *et al.,* 2012; Tang *et al.,* 2017; Turkmen *et al.,* 2019), showing that Roundup evoked histopathological changes in liver and kidney tissue in rats. The protective activity of LuO has been demonstrated in rats with RDP-induced hepatic and renal toxicity. This is due to its high levels of omega-3 and ALA, which can protect against the oxidative damage of liver and kidney cell membranes *via* the free radical scavenging (Yang *et al.,* 2012). Furthermore, a protective effect of linseed has been reported against thiacloprid, cadmium, astaxanthin and hepatic steatosis (Goncalves *et al.,* 2018; Hendawi *et al.,* 2016; Karaca & Eraslan, 2013; Xu *et al.,* 2017).

CONCLUSION

In conclusion, the present study showed that Roundup was able to induce oxidative damage in adult rats by increasing lipid peroxidation, protein carbonylation, and protein oxidation associated with decreased antioxidant enzymatic activity, reduced glutathione content in the liver and kidneys. Given the biochemical effects of RDP in serum, there is a risk of cytotoxicity in farmers and other people who may be in contact with RDP-containing pesticides. Furthermore, the aggravation of inflammation and oxidative stress was confirmed with histological changes observed in our study. The use of linseed oil as an antioxidant proved effective in reducing oxidative stress in liver and kidney and preserved the integrity of tissue structure.

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Interest conflict

The authors reported no potential conflict of interest.

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