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Protective Effect of Essential Oil of Pelargonium Graveolens against Paracetamol Induced Toxicity on Hematological and Hepatic Parameters in Wistar Rats

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Keywords:

Paracetamol; Antioxidant activities; Pelargonium graveolens; Essential oil

Abbreviations:

Paracetamol; P; APAP: acetyl-para-aminophenol BHT: butylated hydroxytoluene; CAT: catalase;; DMSO: dimethylsulfoxide; DPPH: diphenyl-2-picrylhydrazyl; EDTA: ethylenediaminetetraacetic acid; EOC: essential oil of Pelargonium graveolens; FBS: fetal bovine serum; GC/MS: gas chromatography/mass spectrometry; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazoliumbromide; NBT: nitrobluetetrazolium; PBS: phosphatebuffer saline; SOD: superoxide dismutase; TCA: trichloroaceticacid; Tris: 1,1,1-(trishydroxymethyl) aminomethan

1. Abstract

Pelargonium graveolens is an aromatic and medicinal plant, belonging to the Geraniaceae family that grows in temperate areas of the world, which characterized by its therapeutic proprieties. It is widely known as one of the medicinal herbs with the highest antioxidant activity. This study was designed to investigate the antioxidative activity and protective effects of essential oil of Pelargonium graveolense against paracetamol-induced damages in the rat. Thirty six adult Wistar rats were divided into 4 groups of 9 each: (1) a control group; (2) a group of rats who received paracetamol (P) (900 mg/kg) for 4 days, (3) a group fed with essential oil of Pelargonium graveolense then given paracetamol and a group pre-treated with essential oil of Pelargonium graveolense. Our results showed that the chemical characterization of EOPG identified twenty nine compounds representing 98.23% of the total oil. The major compounds from this oil were: z-citral (53.21%), neryl acetate (13.06%), geranyl acetate (10.33%) and graveolensene (4.23%). Our present study has shown a paracetamol poisoning resulted in an oxidative stress evidenced by a significant increase of lipids peroxidation level in hepatic tissueaccompanied by decrease in the activities of SOD CAT and GP .On the other hand, an increased in the levels of serum transaminases (aspartate amino transferase and serum alanine amino transferase), alkaline posphatase, glycemia triglycerides and the level of cholesterol with high disturbance of Hematological indices. The histopathological observations supported the biochemicalevidences of hepato-toxicity. Contrariwise the administration of EOP to rats prevented these alterations and maintained the antioxidant status. In conclusion, our data confirmed that the treatment with essential oil of Pelargonium graveolens is effective in the prevention of complications induced by paracetamol by restoration of liver activities

2. Introduction

The liver is considered one of the most important organ in the body it plays a central role in the regulation of various physiological processes [1]. It is the center of metabolism of nutrients such as carbohydrates, proteins and lipids. It is also involved in the metabolism and excretion of waste metabolites, drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying them [2]. Liver diseases have become one of the major causes of morbidity and mortality, and hepatotoxicity due to drugs appears to be the most common contributing factor [3]. In fact, drug-induced liver injury and acute liver failure occur due to either accidental or intentional overdose of acetaminophen. APAP is an antipyretic and analgesic drug. Paracetamol (acetaminophen) is considered one of the safest drug, it is a widely used as analgesic and anti-pyretic drug with relatively few adverse effects when used at the recommended therapeutic dosage. [4] When used at therapeutic doses, APAP is metabolized by glucuronidation or sulfation by the cytochrome p450 system into the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). Under normal circumstances, NAPQI is rapidly converted to nontoxic metabolites by glutathione (GSH). However, at large doses of APAP, NAPQI levels increase and may react with hepatic proteins, that directly trigger oxidative stress, mitochondrial damage and hepatocellular injury [5]. Acetaminophen is a safe, effective, well-toleratedand cheap analgesic and anti-pyretic drug with relatively few adverse effects when used at the recommended therapeutic dosage. However, it has several side effects in liver.

Liver injury induced by acetaminophen in mice is a commonly used experimental model for screening substances with potential hepatoprotective activity [6]. Growing interest has been observed in the analysis of these natural entities for their potential benefits to human health. Accelerating research of plants used in folk medicine to treat liver diseases and boost liver function has been performed. In this context, Pelargonium graveolens is widely known as one of the medicinal herbs with the highest antioxidant activity [7].Pelargonium graveolens L' Herit is an aromatic and hairy herbaceous shrub, up to 1 m high. Leaves are prickly and carved; flowers are small, usually pink. P. graveolens (geranium) is native to South Africa (Comoros Islands) and it is widely cultivated in Russia, Egypt, Tunisia Algeria, Morocco, Congo, Japan, Central America and Europe (Spain, Italy, France).[8]Essential oils are natural mixtures of terpenes, mainly monoterpenes and sesquiterpenes, which have been increasingly used in complementary therapies because essential oils are usually rich sources of phytochemical mixtures [9]. Essential oils are a folk medicine and recently their use has expanded worldwide to include therapy against various kinds of inflammatory diseases. [10, 11]. Natural products have been increasingly used for the prevention and treatment of various conditions. Geranium essential oil has historically been used in the treatment of dysentery, hemorrhoids, inflammation, heavy menstrual flows and even cancer [12]. The French medicinal community currently treats diabetes, diarrhea, gastric ulcers, liver problems, sterility and urinary stones with this oil [13]. In Chinese homeopathy, the geranium essential oil is known to open up the liver charka and promote the expulsion of toxins that prohibit the achievement of balance within the body. Besides, essential oils and phenolic compounds, have antioxidative properties and may have hepatoprotective properties [14].

To our knowledge, no study has been carried out concerning the protective effects of essential oil of Pelargonium graveolenson APAP-induced liver toxicity and disturbance in hematological indices. Considering the above data, the present study was carried out to assess the protective effect of EOP in APA-induced liver damage in rats.

For this purpose the objectives of the present study are as follows: (1) to elucidate the composition of EOP; (2) to examine the antiradical activity of Pelargonium graveolensusing DPPH radical scavenging assays to measure the total content of phenolics from this plant; (3) to investigate the effect of paracetamol on hepatic tissue and blood profile ; and (4) to evaluate the protective effect of EOP on the oxidative damage caused by this drug.

3. Materials and Methods

3.1. Chemicals

Acetaminphen, Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), Phosphate Buffer Saline (PBS), Antibiotic mixture (streptomycin, penicillin) and trypan blue solution were purchased from Lonza(Cologne Gmbh, Germany)., 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, Folin-Ciocalteus's phenol reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Nitrobluetetrazolium (NBT), Trichloroaceticacid (TCA), Tris,1,1,1-(trishydroxymethyl) aminomethane, Dimethylsulfoxide (DMSO), Methionine, EthylenediamineTetraacetic Acid (EDTA), Riboflavin were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Additionally, other chemicals including solvents such as methanol, hexane and ethanol were used.

3.2. Plant Material

The aerial parts of Pelargonium graveolens were collected during October and November 2016 from the region of SidiAich, Gafsa, Tunisia (Latitude 34.683N, Longitude 8.8E, Altitude 522 m, rainfall 150 mm/year). The collected vegetative parts were air dried at 25°C for 15 days in a shaded and ventilated atmosphere.

3.3. Extraction of Essential Oil

The essential oil sample was extracted from 50 g of dried leaves of the Pelargonium graveolenstreesby hydrodistillation after crushing and immersion in 500 mL of distilled water. The extraction was carried out for three hours. Hexane was used to recover the oil from the extraction apparatus. The organic phase was dried using anhydrous sodium sulphate until the traces of water were totally removed. After filtration, the solvent was evaporated and the essential oil was stored at 4°C in a dark glass bottle until the accomplishment of the phytochemical analyses. [15] The obtained essential oil is called EPG (essential oil of Pelargonium graveolens).

3.4. Experimental Design

3 months-old Wistar male rats, (were obtained from physiology laboratory animal, Gafsa,Tunisia) about 160 g body weight, fed on 15% proteins food pellets (SNA, Sfax, Tunisia), were kept in a

breeding farm, at 22°C, with a stable hygrometry, under constant photoperiod. Animals were treated according to the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes andthe European Convention for the Protection of Vertebrate Animalsused for Experimental and other Scientific Purposes (Council ofEurope No 123, Strasbourg, 1985).The animals were observed for any signs of toxicity, morbidity and mortality for the first 24 h with special attention during the first 4 h.

The experimental rats were divided into four groups of 9 rats each. (C) was the control group, (P) Group consisted of rats treated with acetaminophen, administered orally by gastric intubation in acute dose 900 mg/kg (1/5 DL50 of rats). (EOP + A) group was made up of rats pre-treated with essential oil of Pelargonium graveolens for 42days, and then given acetaminophen for 4 days. EOP group consisted of rats given orally the essential oil of Pelargonium graveolens (65 mg/Kg).

At the end of the experimental period, the rats in each group were rapidly sacrificed by decapitation in order to minimize the handling stress. Blood serum was obtained by centrifugation (1500 x g, 15 min, and 4 C). Liver and kidney were removed, cleaned of fat, weighed and stored at 80 C until use.

3.5. Tissue Homogenate Preparation

0.5 g of the organ was homogenized in 1 mL of tris buffer solution (TBS) using an Ultra-Turax Homogenizer. The extract was then centrifuged (9000 g/15 min at 4°C) and the supernatants were gathered then stored at -80°C.

3.6. Biochemical Essays

3.6.1. Blood and serum parameters: The levels of glycaemia triglycerides of cholesterol, lactate dehydrogenase (LDH), aspartate amino transferase (AST), alanine amino transferase (ALT), and ALPalkaline phosphatase (ALP) in serum were determined by kit methods Spinreact(www.spinreact.com).and the hematological parameters were determined by automatic hematology analyzer (Mindray BC-5800).

3.6.2. Analysis of the level of lipid peroxidation: According to Yagi [16], the lipid peroxidation was estimated by the measurement of the thiobarbituric acid reactive substances (TBARS) including lipid hydroperoxides, aldehydes, and malondialdehyde (MDA). The absorbance was measured at 530 nm. The quantity of TBARS was expressed in nmoles/mg of protein.

3.6.3. The activities of antioxidant enzymes: The method of proportioning the SOD activity using NBT by the anion superoxide O2-is utilized as bases detection of the presence of SOD [17]. The SOD activity of was expressed as U of SOD/mg of protein. The catalytic activity (CAT) was measured at 240 nm by the variation of the consecutive optical density to the dismutation of hydrogen peroxide (H_2O_2). The results were expressed in nmoles of H_2O_2 per min and mg protein [18]. The GPx activity was evaluated according to the method of Flohe and Gunzler [19]. The activity of GPx was expressed in µmoles of GSH oxidized/min/g of protein, at 25°C. The content of proteins in the tissue extracts was measured by the method of Lowry [20] using the bovine serum albumin (BSA) as standard.

3.6.4. Hepatic histology: Liver slices were fixed and included in paraffin. 6 m thick tissue sections were prepared and colored with hematoxyline - eosine. The tissue preparations were observed under an optical microscope (AC 85V-265V). [21].

3.6.5. Gas chromatography/mass spectrometry (GC–MS) analysis: The analysis of the essential oils of Pelargonium graveolenswas performed on a GC–MS HP model 5975B inert MSD (Agilent Technologies, J&W Scientific Products, Palo Alto, CA, USA), equipped with an Agilent Technologies capillary DB-5MS column (30 m length; 0.25 mm i.d.; 0.25 mm film thickness), and coupled to a mass selective detector (MSD5975B, ionization voltage 70 eV; all Agilent, Santa Clara, CA). The carrier gas was He and was used at 1 mL min-1 flow rate. The oven temperature program was as follows: 1 min at 100°C rampedfrom 100 to 260°C at 4°C min-1 and 10 min at 260°C. The chromatograph was equipped with a split/splitless injector used in the split mode. The split ratio was 1:100. Identification of components was assigned by matching their mass spectra with Wiley and NIST library data, standards of the main components and comparing their Kovats retention indices with reference libraries and from the literature [22]

3.7. Phytochemical and antiradical activity of essential oil of plant

3.7.1. DPPH radical - scavenging activity: The free radical scavenging activity of EOC was calculated using method of Blois (1958), Briefly[29], 25µL diluted essential oil at different concentration (0.05, 0.1, 0.15, 0.25, 0.3, 0.4, 0.5 mg/mL) in methanol was mixed with 975 µL of DPPH solution (2,4 mg of DPPH in 100 mL of methanol). After incubation for 30 min at room temperature in the dark, absorption was measured at 517 nm using the blank sample containing the same amount of methanol and DPPH solution, which acted as the negative control and BHT, acid ascorbic, were used as positive controls. Inhibition of DPPH radical was calculated as follows: DPPH scavenging effect (%): ((A-B)/A)*100 where A was the control reaction absorbance and B was the absorbance of essential oil. The experimental tests were carried out in triplicate.

3.7.2. Determination of total phenols content assay: The total amount of phenol content in the essential oil sample was determined according to the method of Kavoosi and Rohmshon[30]by adding 100 µL of essential oil (0.1 mg/mL) to 1 mL Folin-Ciocalteau reagent. After 5 min, 300 µL $NaCO_3$ (10%) was added and the mixture was kept under constant gentle agitation for 1 h. Absorbance was read at 765 nm using a spectrophotometer. Gallic acid was used as standard phenol with concentration (0-0.3 mg/mL). All the experiments were performed in triplicate and the results were calculated as gallic acid equivalent (GAE) from a calibration curve of gallic acid standard solution expressed as mg of gallic acid per 100 µL of essential oil

3.8. Statistical Analysis

Statistical analyses were performed using a software program (SPSS 18 for windows). The comparisons between groups were performed using one-way ANOVA followed by a Tukey post hoc test. $p < 0.05$ was considered statistically significant. The results are presented in the form of mean ± standard error of the mean (SEM).

4. Results

4.1. Chemical Analysis

The chemical characterization of the essential oil using GC-MS identified twenty five compounds accounting 96.4% of the total oil and indicates the occurrence of monoterpenes, sesquiterpenes and diterpenes. The Figure 1 shows the chromatographic profile of the essential oil of Pelargonium graveolens. The identified major compounds wereCitronellol (29,96%), geraniol (20,32%), Geranyl formate (6.5%) and Citronellic acid (4,87%)Table 1 shows the identified constituents, the percentage composition, retention time and retention index.

4.2. Phytochemical Studies

4.2.1. Free radical-scavenging activity of essential oil of Pelargonium graveolens: Table 2a illustrates the antiradical activity of EOC presented by IC50 value, which is defined as the concentration of the antioxidant, required to scavenge 50% of DPPH and calculated by a graph plotting. Free radical scavenging activity of essential oil of Pelargonium graveolens sshowed weak ability to scavenge DPPH free radicals, at least if compared to those of standards (BHT and ascorbic acid). The IC50 value obtained was 0.309±0.018 mg/mL (Figure 2).

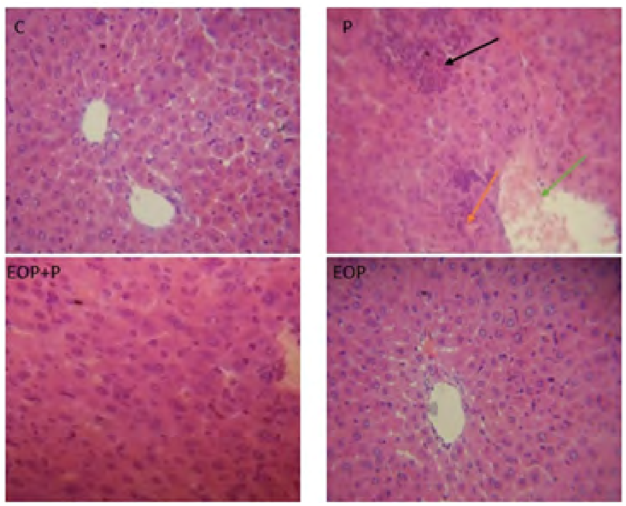

Figure 1: Microscopic observations of rat liver sections (hematoxylin and eosin), (A, B, C and D: 40×; 100×; 400×); (A): control group showing normal hepatic architecture; (B): Paracetamol-treated group showing significant sinusoids congestion, ballooning of hepatocytes, as well as enlargement of nuclei and lymphocytic infiltration in the portal triads and sinusoids; (C): EOP treated rats show normal structure of liver. (D): Paracetamol+EOP showing marked improvement in the section structure of liver.

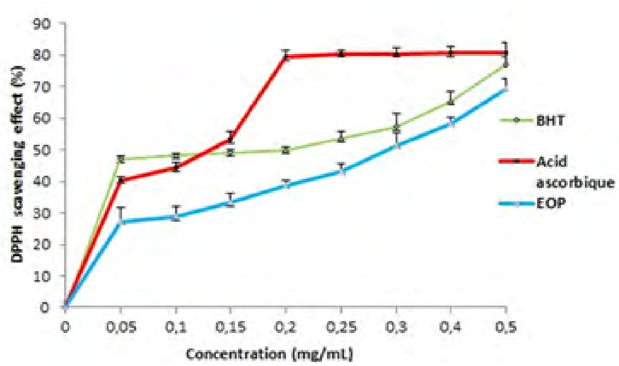

Figure 2: Free radical-scavenging activity of EOP, ascorbic acid and BHT on DPPH. Values are the mean ±S.D. (n=3).DPPH scavenging effect (%)

Table 1:

N°	TR^B	Compound	KIa	Composition
1	7,988	α-Pinene	928	0,58%
2	12,03	p-Cymene	947	0,77%
3	12,504	Graveolensene	953	1,24%
4	12,79	β-phellandrene	998	0,24%
5	12,98	cis-Linalooloxide	1014	0,41%
6	13,02	trans-Linalooloxide	1159	0,64%
7	13.18	Linalool	1199	3,64%
8	13.21	cis-Rose oxide	1356	0,66%
9	13.36	trans-Rose oxide	1356	0,56%
10	7,886	Menthone	1380	2,15%
11	8,195	Iso-Menthol	1489	0 ,13%
12	9,178	α-Terpineol	1306	0,85%
13	9,587	Menthomenthol	1501	2,14%
14	9,654	Citronellol	1533	29,96%
15	9,741	Neral	1523	0,5%
16	10,036	Z-Citral	1554	0,94%
17	10,361	Geraniol	1571	20,32%
18	11,504	Neryl formate	1579	0,76%
19	13,719	Geranyl formate	1580	6,50%
20	14,07	β cariophyllene	1591	1,16%
21	14,287	Methylgeranate	1612	2,81%
22	16,444	Unidentified	1632	0,57%
23	21,338	Citronellicacid	1647	4,87%
24	22,591	α-Muurolene	1659	0,09%
25	23,839	Geranyl butanoate	1679	1,30%
26	24,451	Unidentified	1680	0 ,1%
27	24,461	2-Phenyl ethyltiglate	1706	2,67%
28	25,636	Junenol	1722	0,3%
29	25,743	α-Muurolol	1735	0,18%
30	25,84	α-Cadinol	1745	0,22%
31	26,02	Geranylpentanoate	1766	0,93%
32	26,12	(E)-Citronellyltiglate	1790	0,3%
33	26,43	Unidentified	1800	0,5%
34	26,73	Cadalene	1865	0,33%
35	28,03	cis-Citronellyltiglate	1890	0,6%
36	28,19	Geranyltiglate	1900	3,99%
37	28,17	Geranyl ester	1910	2,72%

Table 2a: Effect of Paracetamol and/or EOP on LDH (U/L), ALP (U/L), AST (U/L), ALT (U/L), Glycaemia (µmoles/L),Cholesterol(mmoles/L)and Triglycerides (g/L) levels in serum

Treatment	C	P	EOP	EOP+P
LDH	1487 ± 304.06	2222.33 ± 204.79*	1451.33 ± 225.08^{+}	1665.5 ± 318.91^{+}
ALP	525±12.73	913± 11.31*	538 ± 28.18^{+}	528 ± 52.33^{+}
AST	283.44 ± 15.21	337.33 ± 18.71*	239.67 ± 18.34^{+}	285.33 ± 21.73^{+}
ALT	87.33 ± 10.41	182.57 ± 38.27*	84 ± 8.89^{+}	108.67 ± 16.17^{+}
Glycaemia	33.78 ± 2.34	48.67 ± 1.15*	32.67 ± 4.51^{+}	36.78 ± 2.09^{+}
Triglycerides	4.87 ± 0.97	8.03 ± 2.08*	4.83 ± 1.82	5.73 ± 1.33^{+}
Cholesterol	62.67 ± 3.21	50.67 ± 13.8	60± 2.65	60.33 ± 16.17

Values are the mean of 9 measurements ± SD; * $p \leq 0.05$: compared to control group (C); + $p \leq 0.05$: compared to Paracetamol-treated group (Paracetamol);

4.2.2. Total Polyphenols: Phenolic compounds are the main agents that can donate a hydrogen atom to free radicals, and thus break the chain reaction of lipid peroxidation, and prevent polyunsaturated fatty acids from oxidative deterioration Here, the total phenolic content of the essential oil of Pelargoniumgraveolens was determined by regression equation of the calibration curve (y=3.131x-0.054, R2=0.997) and expressed in Gallic Acid Equivalent (GAE) per 100 µL essential oil. The total amount polyphenols were about 0.164 mg GAC/100 µL of essential oil.

4.3. Biochemical Parameters

As shown in Table 2b, Paracetamol treatment (900 mg/kg) induced a significant increase of, ALP, ALT, AST, glycaemia triglycerides and the level of cholesterol, whileLDH content did not change significantly as compared to controls. However, the previous supplementation with EOP during 6 weeks seems to protect the fluctuations in those parameters. The previous intake of Pelargonium graveolens in group EOP did not seem to cause any significant change in the biochemical parameters.

4.4. Hematological Parameters

The data in Table 3 shows a significant decrease in the number of leukocytes (WBC) platelets (Plt), the erythrocyte (RBC), count hemoglobin concentration (Hb), hematocrit (HCT),while there is no change of mean corpuscular hemoglobin concentration (MCHC) and mean cell volume (MCV) in Paracetamol-treated rats. The administration ofParacetamol-treated rats with EPG for 6 weeks protected against the alteration of WBC and Pl while the HCT did not seem to be significantly different of Paracetamol-treated group. The treatment of rats with essential oil Pelargonium graveolens alone did not cause any significant alteration in haematological parameters.

Table 3: Effect of Paracetamol and/or EOPon hematological parameters

Treatment	C	P	EOP	P+EOP
WBC	8.89 ± 1.12	5.9 ± 2.41**	8.25 ± 0.49^{+}	7.6 ± 0.85^{++}
RBC	10.32± 0.41	8.35± 0.27 *	10.06 ± 0.33^{++}	9.09 ± 0.55^{++}
Hb	13. 05± 0.78	9.15 ±0.64 **	13.75 ± 0. 57^{++}	12.75 ± 1.06
Plt	966.5± 3.54	378.76 ± 71.32 **	906.89± 56.87^{++}	$^{++}$756.41 ± 35.96^{+}
MCHC	34.6± 0.17	32.8 ± 1.97	34.8 ± 0.22	35.13 ± 0.15^{++}
HCT	37.55 ± 1.91	30.05 ± 0.78**	36.65 ± 2.2^{++}	35.17 ± 2.18^{++}
MCV	46.9± 0.44	47.1 ± 1.44	47.05 ± 0.19	46.9 ± 1.65

Values are the mean of 8 measurements ± SD; Values are the mean of 8 measurements ± SD; * $p \leq 0.01$: compared to control group (C); + $p \leq 0.01$: compared to Paracetamol-treated group (Paracetamol); WBC: white blood cells count (103/µl); RBC: red blood cell count (106/µl); Hb: hemoglobin content (g/dl); Pl: platelet count (103/mm3); MCHC: mean cell hemoglobin concentration (g/dl); HCT: hematocrit (Pg); MCV: mean cell volume (fl).

Table 2b: Radicals' scavenging capacities

Sample DPPH (IC50) ($mg \cdot mL^{-1}$)	
Essential oil of EOP	**0.316±0.025**
BHT	**0.207±0.019**
Ascorbic acid	**0.11±0.037**

Note: Results are the means ± SD of 3 different experiments. BHT, butylated hydroxytoluene; DPPH, diphenyl-2- picrylhydrazyl; EOP, essential oil of Pelargonium graveolens

4.5. Estimation of lipid peroxidation levels (TBARS) in liver extract

Under our experimental conditions, the administration of Paracetamol at a dose of 900mg/kg induced a highly significant increase of hepatic and renal lipid peroxidation compared to control rats which are typical with the hepatic and renal toxicity (Table 4). However, this increase was not detected in the groups receiving Pelargonium graveolens supplementation.

Table 4: Effect of Paracetamol and/or EOP on TBARS level and activities of SOD, GPx and CAT in liver tissue

Treatment	C	P	EOP	P+EOG
TBARS	0.19 ± 0.03	0.48 ± 0.01*	0.2 ± 0.08+	0.29 ± 0.01+
SOD	1.81 ± 0.3	0.84 ± 0.12*	1.78 ± 0.04+	1.33 ± 0. 19+
GPx	15.52 ± 1.01	10.18 ± 0.62*	14.43 ± 2.7+	13.97 ± 0.87+
CAT	11.09 ± 0.75	5.67 ± 0.72*	10.28 ± 1.71+	9.74 ± 1.7+

Values are the mean of 6 measurements ± SD; Values are the mean of 8 measurements ± SD; * $p \leq 0.05$: compared to control group (C); + $p \leq 0.05$: compared to Paracetamol-treated group (Paracetamol); TBARS: Lipid peroxidation level (nmoles MDA/mg proteins); SOD: superoxide dismutase (U of SOD/mg proteins); GPx: glutathione peroxidase (µmoles of oxidized GSH//min/mg proteins); CAT: catalase (µmoles of transformed H2O2/min/mg proteins)

4.6. Changes of antioxidant enzyme activities in liver extract

The results represented in Table 4 shows that the treatment with acetaminophen resulted in a significant increase in SOD, GPx and CAT activities in liver. However, no significant effect of paracetamol was observed when it is associated with Pelargonium graveolens oil supplementation.

4.6.1. Histopathology Examination: The observation of histological sections of liver from control rats (Figure 1A) shows a normal cellular structure of the liver tissue with well-designed hepatocytes radiating from central vein separated by blood sinusoids and the hepatocytes contains central pale stained nuclei. However, for rats treated with APAP, histological sections showed significant congestion of the sinusoids (coagulative necrosis) which became ill-defined. In the liver of this group there is also an enlargement of the hepatocytes and vacuolization (in the cytoplasm, as well as enlargement of nuclei (dark stained). Indeed, there is a remarkable leukocyte infiltration in the portal triads (triaditis) and sinusoids all around the foamy of hepatocytes as a sign of inflammation (Figure 1B). The liver sections in rats treated with Pelargonium graveolens essential oil , showed a good recovery with less necrosis.

5. Discussion

Natural products have important biological properties in disease prevention as in hepatoprotective capacity. This activity of natural products can be explained by its antioxidant properties deriving from monoterpenes, flavonoids, and phenols [23]. Pelargoniumspecies are the most popular fruits containing essential oils, citric acid, ascorbic acid, carotenoids, and mineral.These substances prevent damage to cell membrane and other structures by neutralizing free radicals [24] Then, the phenolic acids and flavonoids have been proven to be able to (1) liberate hydrogen proton from their hydroxyl group, (2) scavenge free radicals; and (3) prevent cells from oxidative damage.

Moreover, Pelargonium oil generally contains over 90% of monoterpenes, about 6% of oxygenated compounds and less than 1% of non-volatiles [25]. Upon GC/MS analysis, the EOP was found to contain 37 constituents eluted from 8 to 30 min, accounting for 96.4% of the essential oil were identified (Table 1). All the volatiles were monoterpenes, sesquiterpenes and diterpenes, both hydrocarbons and oxygenated derivatives. Compared with other studies we found abundant monoterpenoids (80.90%) in the AEO. The results showed that the most abundant constituent of EOPis Citronellol (29,96%), other major components of EOP include geraniol (20,32%), Geranyl formate (6.5%) and Citronellic acid (4,87%).

These results were quite different with previous studies [26] which demonstrated that the geranium leaves essential oil contains high levels of these compounds.in fact, various reports have shown that the level of linalool was up to 10% and geraniol is reduced to 6%in EOP[26] (Farukh) [27]. Furthermore, lowest concentrations of Citronellic acid and geranyl formate were detected in EOP The variation in the chemical composition may be related to the environmental conditions that the plant was exposed to, such as mineral water, sunlight, the stage of development and nutrition. It was observed by Fayed et al [28] that under the same climatic conditions, the composition of rose-scented geranium essential oil was significantly affected by crop duration and length of vegetation period. Further, BenHsouna and his collaborators [29] were proved that the antioxidant activity of the essential oil could be attributed in part to the presence of compounds such as Citronellol and geraniol and its ability to decompose free radicals by quenching reactive oxygen species and trapping radicals before reaching their cellular targets. To detect the antioxidant capacity of our essential oil, we have used the DPPH test that has been widely used to determine the free radical scavenging capacity of various samples because of DPPH stability, simplicity, and fast assay [30]. In fact, the present study demonstrated that EOP had moderate antiradical activity with IC50 lower that the synthetic antioxidants used as BHT (IC50= 0.20 mg/mL) and ascorbic acid (IC50= 0.13 mg/mL).

These results are in agreement with those reported in study of Džamić [31] which showed that the essential oils of Pelargonium graveolens had much lower antioxidant activity resulting in DPPH inhibition percentages of 25.19%, while BHT yielded activity reaching 85.42%. Comparing this Pelargonium graveolens oil activity with other DPPH scavenging activity of essential oil Pelargonium graveolens from Serbia and Egypt respectively ,we found that IC50 of EOP than in essential oil (IC50 = 0.802 µg/Ml, IC50 = 0.468µg/mL) . However, the IC50 of EOP is higher than in essential oil of Pelargonium from Spain (IC50 = 1.80 mg/mL) [32]. Therefore, The antiradical scavenging activity of the oil might be attributed to the replacement of hydroxyl groups in the aromatic ring systems of the phenolic compounds as a result of their hydrogen donating ability and thus stopping the chain reaction of lipid oxidation at the initial step. Based on the obtained data, it can be suggested that the differences in antioxidant activities of essential oils of different Pelargonium species may differ depending on the type and source of plant material [33].

In addition, the essential oil of Pelargonium graveolens contained moderate amount of phenolic compounds (0.146±0.001 mg GAC/100 µL essential oil). This level of phenolic compounds of Pelargonium was lower than in essential oils found in other plants as Artemisia arborescens (0.163± 0.004 mg GAE/100 µL of essential oil) [34],. According to previous studies, it seems that antioxidant activities of essentials oil might be due to their richness in bioactive molecules that operate as therapeutic agent [35].

Besides, the results in the present study showed that the administration of paracetamol at 900 mg/kg dose (group P) to rats caused destruction of liver cells in turn resulting in the elevation in serum level of enzymes aminotransferases. An obvious sign of hepatic injury is the leaking of cellular enzymes such as ALT, AST and ALP into plasma due to the disturbance caused in the transport functions of hepatocytes. ALT is more specific to the liver, and it is a better parameter for analyzing hepatic injury. High levels of AST indicate the cellular leakage as well as loss of functional ability of cell membrane in liver. Serum ALP is also related with liver cell damage. High concentration of ALP cause serious hepatic damage in paracetamol treated rats. Therefore, serum hepatic biomarkers analysis is important for identification of liver lesion [36]. Also The levels of serum of glucose, cholesterol, triglycerides and the level of cholesterol that were significantly increased compared to their corresponding values in the control group (C). These findings are in agreement with those reached by El-Sayed et al [37]. They found that the administration of acetaminophen aspirin at a high dose for 3 days in rats resulted in significant elevation of total cholesterol, glycaemia and aspartate transaminase activity. Such high dose (850 mg/kg) of aspirin has been reported to cause also damage in other organs;, Also, our results are similar to those reported in the study of Zhang et al [38] which proved that analgesic such acetaminophen, may alter the function of theliver's, causing elevation of serum aspartate as well as alanineaminotransferases and necrosis of hepatic cell.

Paracetamol hepatotoxicity was evidenced also by disorder in the blood profile. In fact, in blood, the administration of acetaminophen induced a significant decrease of platelet leukocytes,the erythrocyte count hemoglobin concentration (Hb), and hematocrit which can be explained by damages affecting the hematology function. The alteration of WBC and platelet may be due to an excessive storage of platelets and WBC by the spleen [39]. Overall it appears the effect of the paracetamol resulted in a drop in RBC, Hgb, MCV and HCT [40] This can reveal anemia caused by the high dose of paracetamol .Greene and Hagemann considered paracetamol one of the Over 130 drugs have indisputable evidence of causing haemolytic anaemia [41]

On the other hand, our study showed that the administration of aspirin induced significant increase of lipid peroxidation (TBARS) in the liver and the kidney by about 108% and 55.17%, respectively, compared to the control. The increase of TBARS was confirmed by the peroxidative effect of aspirin. Our results confirmed recent findings [42] showing that paracetamol induced an oxidative stress in liver and kidney of rats by decreasing the activities of antioxidative enzymes (SOD, CAT, GPx) when compared with normal control rats. Li et al. [43] showed that APAP induced an oxidative stress in mice. The decrease in the level of SOD (the enzyme responsible for the dismutation of superoxide to hydrogenperoxide) led to excess superoxide radicals and organic peroxides by generation of highly reactive entities, which results in an attackon DNA, membrane lipids and other essential cell compounds. Then, the decrease in CAT activity, i.e. the enzyme involved in the decomposition of H2O2 to water and oxygen, leads to a reduction in glutathione content as well as exacerbation of free radical production [44]

Antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) are very important in protecting organisms from reacting oxygen species. SOD is a defense enzyme, which converts superoxide radicals to hydrogen peroxide. Catalase is a hemeprotein found in peroxisomes of eukaryotic cells that catalyses the conversion of hydrogen peroxide to water and oxygen. [45] GPx plays a critical role in maintaining balance in the redox status of animals under acute oxidative stress and protect against chemically induced oxidative destruction of lipid and proteins.Indeed, our study showed that the administration of paracetamol induced significant increase of lipid peroxidation (TBARS) in the liver. [46] The increase of TBARS was confirmed by the peroxidative effect of aspirin. Lipid peroxidation has been postulated to be the destructive process in liver injury due to paracetamol administration. The increase in MDA level of liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. The decrease of glutathione, GPx, SOD and catalase enzyme activity may indicate the toxic effects of reactive oxygen species produced by acetaminophen.Our results confirmed recent findings [47] showing that Paracetamol induced an oxidative stress in liver and kidney of rats by decreasing the activities of antioxidative enzymes (SOD, CAT, GPx) when compared with normal control rats. Reduced GSH level was depleted in paracetamol treated group may be due to conjugation of glutathione with NAPQ1 to form mercapturic acid. In fact High doses of APAP, the oxidation pathway is initiatedby the formation of the reactive metabolite NAPQI,which is generated mainly by the cytochrome P450 enzymesCyp2e1 in mice and humans [48]. Excessive NAPQI formation after APAP overdose depletes cellular glutathione, adducts proteins, including mitochondrial proteins, and induces mitochondrial oxidant stress and dysfunction; this results in nuclear DNA fragmentation and in massive necrosis and apoptosis of hepatocytes. [49]. Toxic overdose of paracetamol depletes hepatic GSH content so that free NAPQI binds covalently to cellular mitochondrial proteins which suppresses mitochondrial fatty acid β-oxidation and results in massive necrosis and apoptosis of hepatocytes also it has been suggested that the decrease in the levels of enzymatic antioxidants (SOD, CAT, GPx) was observed in APAP-treated rats and it could be due to reduction in their biosynthesis or their excessive utilization in trapping the free radicals generated. [50].

In addition, the toxic effects of APAP, proved by biochemical and haematological findings, were also confirmed by histological observation showing centrilobular vein congestion, presence of inflammatory cell infiltration, and vacuolization of hepatocytes. These data were confirmed by Ben Slima [51]. et al indicating that the administration of essential prevented hepatic alterations.

In general, the hepatoprotective activity of plants can be considered as an expression of the functional improvement of hepatocytes that results from accelerated cellular regeneration Therefore, EOP that has been employed as a protective treatment of liver damage by its antioxidant properties deriving from the phenolic nature of Pelargonium graveolens.In this context, our results showed that pre-treatment with EOP was able to reduce levels of AST, ALT, ALP, glucose, cholesterol and triglycerides at all doses employed, improving liver damage when compared with the APAP-treated rat. The liver histopathological analysis in groups pre-treated with essential oil of Pelargonium showed hepatocytes preserved, infiltrated cells equivalent to normal, and the morphology of the hepatic parenchyma similar to the control group, suggesting a protective effect of EOP. The potent benefic effect of EOP might be related to the high level of phytochemical compounds.

The decrease of the activities of liver enzymes in blood and the improvement of hepatocellular architecture with signs of recovery, due to its ability to reduce free radical- induced oxidative damage in the liver. These findings are in agreement with those reported by Boukhris [52] who showed that administration of EOP ameliorated the toxicity of alloxan by restoring the levels of glucose. The studies conducted by Elsayed et al. [53] demonstrated that the treatment of Pelargonium graveolensextract in rats reduced AST, ALT and ALP.

In addition, the treatment of essential oil exerted a strong protective effect on APAP-induced oxidative stress, as revealed by the decreased level of lipid peroxidation (TBARS), and enhanced the enzymatic defense system (SOD, CAT and GPx). Our results are confirmed by Lis-Balchin et al. [54] who showed that the essential oil of Pelargonium graveolens exhibits an antioxidant action in preventing lip-operoxidation. Also, these results are in agreement with results found by Peterson et al . [55] who suggeste that EOP significantly restored the hepatic GSH level, That could scavenge the reactive free radicals that eventually reduced the oxidative damage to the tissues and subsequently improved the level of this antioxidant These cellular oxidative/antioxidant factors play animportant role in maintaining the redox homeostasis under normal physiological conditions. APAP treatment depleted the glutathione level and caused oxidative stress and redox imbalance. The essential oil treatment significantly prevented the decline in the activities of the oxidative stress/antioxidant parameters altered due to APAP administration [56].The mechanism of hepatoprotection by EOP of leaves is due to their antioxidant potential. This suggests that leaf extracts can reduce ROS that may lessen the oxidative damage to the hepatocytes and improve the activities of the liver antioxidant enzymes, thus protecting the liver from paracetamol induced damage. Also, the possible mechanism could be by the stimulation of hepatic regeneration through an improved synthesis of protein or accelerated detoxification and excretion.

Mativandlela [57] demonstrated the hepatoprotective effect juice of Pelargonium against paracetamol induced liver injury in rats. Due to the fact that lemon contains a variety of bioactive ingredients. The antioxidant properties of of geranium essential oil a were attributed to to the presence of monoterpenes. The Citronellol andgeraniol, were the major monoterpenes detected in the chemical composition of geranium oils, are all known to be efficient radical scavengers [58]. Moreover, the measured antioxidant activities could be due to the synergistic effects of two or more compounds present in the oils. In this context, Lu and Nivitabishekam et al. [59] reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative.

6. Conclusion

Our study indicates that chemical composition of geranium oil is of high quality with citronellol and geraniol as dominant compounds. The oil expressed stronger hepatoprotective effect as it restore all the liver function and oxidative stress markers to the desirable levels. This is aided by the superior antioxidant potential of P. graveolens. against the sequential events of free radical toxicity by paracetamol. It can be concluded from the observations in our study that EOP may have a protective effect against paracetamol induced hepatotoxicity in rats these observations prompt that The essential oil Pelargonium gravelones and its main component possess a wide spectrum of biological activity, which may be of great importance pharmacology and pharmaceutics.

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