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Research article

Rosemary and parsley extracts minimize Isoniazid®-induced hematological deterioration and enhance the oxygenation potential in adult male albino rats

Khaled G. Abdel-Wahhab[1*], Abdel-Baset M. A. Abd El-Reheem[2], Mohammed M. Abdel-Wahhab[2,3], Salah M. E. Soliman[2], Mahmoud Ashry[2]

[1]*Medical Physiology Department, National Research Centre, Egypt.*
[2] *Zoology Department, Faculty of Science, Al-Azhar University, Egypt.*
[3]*Department of Biology, Faculty of Science, Jazan University, Kingdom Saudi Arabia.*

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***Corresponding Author: Khaled G. Abdel-Wahhab,** Medical physiology department, National Research Centre, Egypt.

Email: kgm194@yahoo.com
Phone No:+201110917108

Abstract

Isoniazid® (INH), being the first line drug used as anti-tuberculosis drugs, is known to be associated with physiological deteriorations including hematological disturbances. The objective of this study was to explore the protective effect of rosemary and parsley aqueous extracts against INH-induced hematological disturbances. Adult rats (120-150g) were randomly divided into six groups (10 rats each): first group administrated with saline and served as control, second group ingested rosemary extract (440mg/kg/day), third group ingested parsley extract (250 mg/kg/day), fourth group received Isoniazid® (50mg/kg/day), fifth group received Isoniazid® and rosemary extract together, and sixth group received Isoniazid® in combination with parsley extract. After eight weeks, the results revealed that administration of either rosemary or parsley extract in combination with Isoniazid® ameliorated the Isoniazid®-induced hemato-deterioration; this was evidenced by the significant improvement of blood Hb, RBCs, HCt, blood indices, TLC, platelets and oxyhemoglobin (Hb-O_2, functional Hb derivative) levels and met-Hbr activity coupled with a reduction in the level of nonfunctional Hb derivatives (met-Hb, Hb-CO and Hb-S), auto-oxidation rate of oxyhemoglobin and hemolysis of RBCs. In conclusion, both rosemary and parsley extract could play a beneficial role in prevention of Isoniazid®-induced hematological disturbances, consequently reducing both physiological and functional anemia. This effect could be through their anti-oxidative and anti-nitrosative voltage.

Introduction

Drugs used for the treatment of tuberculosis have been reported to cause major adverse reactions and significant morbidities leading to a compromised treatment regimen [1]. Several studies had reported that leukopenia, eosinophilia, hemolytic anemia along with hepatotoxicity, fatigue, dizziness, headache and dyspnoea occurred after Isoniazid® administration [2-3]. Isoniazid® (INH) is a synthetic chemical and a pyridine derivative of nicotinamide; the central nervous system, liver, and hematological systems are the main targets of INH toxicity. Acutely it may cause leukocytosis, and chronically it may determine anemia (hemolytic, sideroblastic, aplastic, or megaloblastic), agranulocytosis, eosinophilia, or thrombocytopenia; disseminated intravascular coagulation and lymphadenopathy due to hypersensitivity reactions have also been reported [4].
Natural components behave and include different functional activities, for instance, antioxidant activity [5], antimicrobial activity [6], anti-hypertensive [7], anti-cancer [8], or neurodegenerative diseases prevention [9-11]; Parsley and Rosemary extracts are among them and are rich in phytochemical derivatives such as triterpenes, flavonoids or polyphenols; many studies reported that the preventive effects of rosemary or its extracts are attributed to its antioxidant activity [12-14]. It was reported that carnosol, rosmanol and epirosmanol phenolic diterpenes of rosemary inhibit lipid peroxidation; additionally, ursolic acid, a constant constituent of *Rosmarinus officinalis* extracts, has been shown to have antioxidant and anticarcinogenic properties [15]. Rosmarinic acid exhibits antioxidant, anti-inflammatory effects, hepatoprotective effect, nephroprotective effect and hematoprotective effect [16]. Rosemary extracts are able to donate electrons to reactive radicals, converting them to more stable structures, therefore preventing them from reaching the biomolecules in susceptible biological systems. Also, it was concluded that rosemary extracts have a high scavenging capacity of different types of reactive oxygen and nitrogen species, is thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals [17]. Parsley *(Petroselinum crispum*) leaves were reported to be used for treatment of hematological disorders, constipation, flatulence, jaundice, colic, edema, and rheumatism as well as diseases of prostate and liver. It has also been used as an

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aphrodisiac [18]. Parsley is a good source of iron, calcium, phosphorous and antioxidants like luteolin, vitamin C, vitamin A and zinc, which may account for its hepatoprotective effect and hematoprotective effect [19, 20]. As a large number of herbs has been traditionally used to treat or reduce drug-induced complications, therefore the main objective of the present study was to explore the protective and ameliorating battery of both rosemary and parsley aqueous extracts against INH-induced hematological deteriorations (physiological and functional anemia) in a trial to enhance the oxygen transport and delivery consequently improving the drug efficacy and the body tolerance in tuberculosis patients..

Materials and methods

Herb extraction

Rosemary (*Rosmarinus officinalis*) and parsley (*Petroselinum crispum*), herbs were obtained from a local supplier, (Bab El-Khalk zone, Cairo, Egypt) identified and authenticated by scientific botanists at Botany Department, Faculty of Science Al-Azhar University and they were found to have taxonomic serial numbers (TSN) 32677 and 29817 respectively. The extraction process of the dry leaves was carried out according to the method of Gulcin *et al.* [21]. In brief, 100 g of the powdered herb leaves were placed in a 1000 ml round-bottom quick fit flask, and 400 ml distilled water were added; the mixture was left for 24 hours at 8 °C, and filtered through qualitative Whatman filter paper No.1. Then, the filtrate was subjected to lypholyzation process through freeze drier (Snijders Scientific-tilburg, Holland) under pressure, 0.1 to 0.5 mbar and temperature -35 to -41°C conditions. The dry extract was stored at -20°C until used. The yield, total phenolic content and radical scavenging activity of the obtained extract were investigated.

Total phenolic content (TPC)

The concentration of total phenolic content in both herb extracts was determined using the method of Jayaprakasha *et al.* [22] and the results were expressed as catechin equivalents (CE). 5 mg of the extract was dissolved in a 10 ml of acetone/water mixture (6:4 v/v); samples of 0.2 ml of that solution (50% w/v) was mixed with 1.0 ml of Folin-Ciocalteu (10-folds diluted) reagent and 0.8 ml of sodium carbonate solution (7.5%); after 30 minutes at room temperature, the absorbance was measured at 765 nm using UV–160 1PC UV-visible spectrophotometer. Estimation of phenolic compounds as catechin equivalents (CE) was carried out using standard curve of catechin.

Radical scavenging activity (RSA) by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The capacity of antioxidants to quench DPPH radical was determined according to Nogala-Kalucka *et al* [23] method. Certain amount of crude extract was dissolved in methanol to obtain a concentration of 200 ppm; then 0.2 ml of this solution was completed to 4 ml by methanol, and 1 ml of DPPH (6.09 x 10^{-5} mol/L) solution in the same solvent was then added. The absorbance was measured after 10 min at 516nm against reference blank which was 1ml of DPPH solution and 4 ml methanol. RSA was calculated according the formula.

$$\text{RSA}\ (\%) = \left(\frac{\text{A}_{\text{control sample}} - \text{A}_{\text{sample extract}}}{\text{A}_{\text{control sample}}}\right) * 100$$

Animals and experimental design

Adult male Wistar albino rats (*Rattus norvegicus*) weighting 120-150g were obtained from Animal colony, National Research Centre, Dokki, Egypt. The animals were housed in suitable plastic cages one week for acclimation before start the experiment. Fresh tap water and standard rodent food pellets (Agricultural-Industrial Integration Company, Giza, Egypt) were always available. All animals received human care in compliance with the standard institutional criteria for the care and use of experimental animals according to the ethical committee of National Research Centre, Egypt (FWA00014747). After animals being acclimatized with the experimental room conditions, they were randomly divided into six groups (10 animals each); group (I) normal rats daily administrated (0.4 ml//kg b.wt) saline by oral intubation for eight weeks and acted as control, group (II) animals subjected to daily oral administration of rosemary aqueous extract (440 mg/kg b.w) for eight weeks according to Amin and Hamza [24], group (III) animals subjected to daily oral administration of parsley aqueous extract (250 mg/kg b.w) for eight weeks according to Pourush *et al.* [25], group (IV) animals subjected to daily oral administrated with INH (50mg/kg b.w) for same period [26], group (V) animals subjected to daily oral administration of INH together with rosemary aqueous extract a similar period, and finally group (VI) animals subjected to daily oral administration of INH in combination with parsley aqueous extract for a similar period.

Blood sampling

At the end of the study period, animals were fasted overnight and following diethyl ether anesthesia heparinized blood was withdrawn from the retro-orbital plexus using capillary sterile glass and heparinized tube (single draw vacutainer needle) into open vacutainer collecting tubes for hematological measurements.

Complete blood count

Cell blood counter (full automatic –Model PCE – 210 N, Japan) was used for measuring of red blood corpuscles (RBCs) count (10^6/cm^3), Hemoglobin (Hb) content (g/dl), hematocrite (Hct) percentage, mean corpuscular volume

(MCV) (fl), mean corpuscular hemoglobin (MCH) (pg), mean corpuscular hemoglobin concentration (MCHC) (g/dl), platelets (PLT) count ($10^3/cm^3$), and total leucocytes count (TLC) count ($10^3/cm^3$).

Non-functional hemoglobin derivatives

Methemoglobin (met-Hb)

Blood methemoglobin (met-Hb) level (as % to total Hb) was determined in whole heparinized blood using the colorimetric method described by Evelyn and Malloy [27]; exactly 0.2ml of the heparinized blood was lysed vigorously in 10 ml of a solution containing 4ml of the freshly prepared phosphate buffer and 6ml of non-ionic detergent solution 1%. After centrifugation at 1000g for 10 minute; the clear lysate (supernatant) was divided into two equal volumes (A&B). The absorbance (D1) of the volume A was measured at 630nm, then one drop of potassium cyanide solution was added and the absorbance (D2) was measured again at 630nm after mixing. One drop of potassium ferricyanide was added to the volume (B) and the absorbance (D3) was measured at 630nm after 5 minutes, then one drop of potassium cyanide was added and the absorbance (D4) was measured at 630nm immediately after mixing. All measurements were made against the reagent blank (buffer and the non - ionic detergent in the same proportion in sample). Mmethemoglobin % is then calculated according to the formula below.

$$\text{met-Hb (\%)} = \frac{D1-D2}{D3-D4} \times 100$$

Sulf-hemoglobin (Hb-S) level

Sulf-hemoglobin (Hb-S) level (as % to total Hb) was determined in whole anti-coagulated blood using the colorimetric method described by Van Kampen and Zulstra [28]. Mixing 0.1 ml of whole blood with 10 ml of non-ionic detergent (2%) solution, and then the absorbance (A) at 620 nm was measured. After that, one drop of potassium cyanide (5%) was added and r the absorbances were recorded at 620 nm and 578 nm after 5 minutes. The percentage of S-Hb was calculated from the equation S-Hb % = 2× A_{620} (S-Hb)/ A_{620} (Hb-O_2), where, A_{620} Hb-O_2 = A_{578} / conversion factor, and A_{620} S-Hb = A_{620} total Hb - A_{620} (Hb-O_2).

Carboxy-hemoglobin (Hb-CO) level

Carboxy-hemoglobin (Hb-CO) level (as % to total Hb) was evaluated using the method described by Van Assendelft [29]. Exactly, 0.1 ml of whole blood was diluted in 20ml of ammonia (0.4ml/l) and 20mg sodium dithionite was added. Within 10 minutes, the absorbencies at 538nm and 578 nm were recorded spectrophotometrically. The Hb-Co level was calculated from the equation Hb-Co (%) = [2.44× (A_{538}/A_{578})] – 2.66.

Functional hemoglobin derivative

Oxy-hemoglobin (Hb-O_2) level

As the four derivatives representing 100% to total Hb, therefore Hb-O_2 (%) could be calculated mathematically from the formula Hb-O_2 (%) = [100 – Met-Hb (%)–S-Hb (%) – Hb-Co (%)].

NADH-Methaemoglobin reductase activity of erythrocyte lysate

NADH-Methemoglobin reductase (NADH-MR) activity of erythrocyte haemolysate was assayed according to the method of Board *et al.* [30]. The reaction mixture contained 0.2ml Tris-HCl/EDTA buffer pH=8.0, 0.2ml NADH and 0.35ml of distilled water, 0.2ml of $K_3Fe(CN)_6$ and 0.05ml of erythrocyte haemolysate. The 0.2ml tris-HCl/EDTA buffer pH=8.0, 0.2ml NADH and 0.35ml of distilled water were introduced into a test tube and incubated for 10minutes at 30^{O}C. The mixture was transferred into a cuvette and the reaction was started by adding 0.2ml of K3Fe (CN) 6 followed by 0.05ml of erythrocyte haemolysate. The increase in absorbance of the medium at 30OC was followed spectrophotometrically at 340nm for 10 minutes at 60 seconds intervals against a blank solution. The equation below was used to evaluate erythrocyte NADH-MR activity in international unit per gram hemoglobin (IU/gHb).

$$\text{Enzyme activity (IU/gHb)} = \frac{100}{[\text{Hb}]} \text{x} \frac{\Delta A}{\sum} \text{x} \frac{Vc}{Vh}$$

Where, [Hb] is hemolysates' hemoglobin concentration (g/dl), ΔA is the change per minute in absorbance at 340nm, ∑ is millimolar extinction coefficient which equals 6.22 of a reaction in which 1mole of NADH + H^+ is oxidized, VC is the cuvette volume or total assay volume that equals 1 ml, and V_h is the volume of haemolysate in the reaction system which equals 0.05ml.

Auto-oxidation rate of Oxy-hemoglobin

Auto-oxidation rate of Hb-O_2 was determined according to the method described by Mansouri and Winterhalter [31]. Freshly obtained heparinized blood samples were centrifuged at 3000 rpm for 10 minutes. After removal of the plasma and buffy coat, the red blood cells (RBCs) were washed three times with 10 folds ice cold NaCl (0.9%) solution and repeat the centrifugation. The packed RBCs were vigorously hemolyzed by the addition of 5 volumes of ice cold distilled water and re-centrifuged at 10.000 g for 20 minutes, for removing of the erythrocytes ghosts and membranes. Stock hemoglobin solution was finally prepared. Hemoglobin solution was diluted with ice cold distilled water to obtain diluted Hb solution with an optical density at 578nm equals one (A578 = 1.0). The diluted blood samples were incubated at 37°C and the

absorbance at 630nm was measured at zero, 60, 120, 180, 240, and 300 minute. The obtained absorbance data were plotted statistically against the time and the auto-oxidation rate of Hb was calculated from the linear equation.

Osmotic fragility

The percentage of hemolysis was assessed under stress of HgCl according to the modified method described by Nicak and Mojzis [32]. Erythrocyte hemolysis was determined using a series of eleven different osmotic NaCl saline composed that of H_2O. The percentage of hemolysis was calculated according to the following formula: Hemolysis % = (A sample/A H_2O)*100.

Statistical analysis

The obtained data were subjected to one way ANOVA followed by post hoc (Tukey) test using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA. The significance between the means was tested at $p \leq 0.05$ [33].

Results

With regard to the *in vitro* investigations; the mean values of yield, radical scavenging activity (RSA) and total phenolics content (TPC) of the aqueous extracts of both tested herbs are illustrated in figure (1). The obtained data revealed that rosemary extract (RE) possesses values of yield, RSA and TPC higher than those of parsley extract.

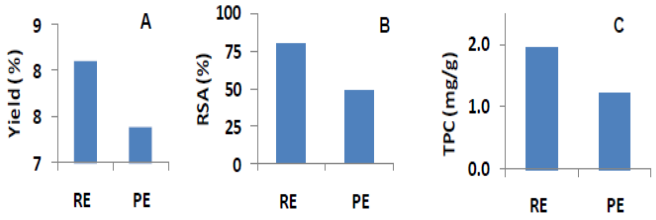


Figure 1. Mean values of three replicates of yield (A), radical scavenging activity (B) and total phenolic content (C) of both rosemary and parsley aqueous extracts.

Animals those were administrated orally with the aqueous extract of ether rosemary or parsley didn't show any unfavorable changes in whole blood Hb, RBCs, HCT, MCV, MCH, MCHC, PLT and TLC, while animals group that treated with Isoniazid® only revealed a significant reduction in Hb, RBCs, HCT, MCH and MCHC resulted in hypochromic or physiological anemia; also it revealed a significant reduction in PLT count or thrombocytopenia and total leukocytes count or leukocytosis when the three groups were compared to normal animals. Fortunately, animals groups those were treated with the aqueous extract of either rosemary or parsley along with Isoniazid® showed a significant improvement mostly in the measured blood parameters, when all were compared corresponding values of Isoniazid®-treated rats' group.

Table 1. Mean values of whole blood Hb, RBCs, MCV, MCH, MCHC, HCT, PLT and TLC of treated and control male Wistar albino rats.

	Control	RE	PE	INH	INH+RE	INH+PE
Hb% (g/dl)	17.2±1.5 ^{a}	17.3±1.5^{a}	16.9±0.3^{a}	10.8±0.4^{c}	15.2±1.4^{d}	14.4±0.5^{d}
RBCs (10^6/ cm^3)	9.4±0.8 ^{a}	9.5±0.59^{a}	9.3±0.42^{a}	7±0.86^{c}	9.5±0.71^{d}	9.4±0.32^{d}
HCT (%)	49.5±4.7 ^{a}	53.3±5.7^{a}	50.4±2.4^{a}	38.6±4.5^{c}	55.9±4.5^{d}	53.4±1.2^{d}
MCV (fl)	52.1±0.76 ^{a}	55.3±1.35^{a}	54.9±0.57^{a}	53.8±0.96^{a}	54.6±1.7^{a}	52.7±1.5^{a}
MCH (pg)	18.2±0.9	18.1±0.7^{a}	17.9±0.9^{a}	15.2±0.9^{c}	16.9±0.6^{b}	16.6±0.9^{b}
MCHC (g/dl)	35.1±1.5 ^{a}	35.5±0.43^{a}	32.7±1.7^{a}	30.6±0.48^{c}	33.3±0.34^{b}	30.9±0.82^{c}
PLT (10^3/cm^3)	867±83 ^{a}	895.6±90^{a}	856.2±110^{a}	465.2±125^{b}	762±30^{d}	640±85^{c}
TLC (10^3/ cm^3)	10.8±0.83	10.4±1.03^{a}	11.2±1.4^{a}	3.9±0.52^{c}	7.7±0.82^{b}	6.5±1.6^{b}

All data are presented as mean ± standard error. Within each row, means with different superscript letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by post hoc test (Tukey).

In addition, administration of rosemary and parsley extracts for duration of eight weeks showed a slight lowering potential on the level of the non functional Hb derivatives [met-Hb, Hb-CO and Hb-S] matched with insignificant or minute rise in functional derivative [Hb-O_2] and met-Hbr activity. On the other side, Isoniazid®-treated animals showed a significant elevation in non-functional Hb derivatives [met-Hb, Hb-CO and Hb-S] level, coupled with a significant reduction in the functional Hb derivative [Hb-O_2] level and met-Hbr activity, resulting in an additional type of anemia which is functional anemia, when compared to control group. Favorably and with respect to group of animals treated with Isoniazid® only, animals groups received Isoniazid® along with either rosemary or parsley extract showed a significant reduction in the percentage of these non-functional Hb derivatives [met-Hb, Hb-CO and Hb-S] coupled with an enhancement in percentage of the functional Hb form [Hb-O_2] and the activity of the reducing enzyme [met-Hbr] (Table 2 and figure 2).

Table 2. Mean values of Hb-O_2, met-Hb, Hb-CO and Hb-S levels of treated and control male Wistar albino rats.

	Control	RE	PE	INH	INH+RE	INH+PE
Hb-O_2 (%)	97.39±0.056	97.58±0.126^a	97.86±0.100^a	88.04±1.32^c	92.41±0.76^b	91.09±0.92^b
Met-Hb (%)	1.56±0.05	1.49±0.14^a	1.51±0.06^a	9.2±1.27^b	5.21±0.72^e	6.35±0.88^e
Hb-CO (%)	0.58±0.01	0.58±0.09^a	0.59±0.07^a	1.12±0.17^b	0.89±0.15^e	0.96±0.16^e
Hb-S (%)	0.44±0.012	0.39±0.093^a	0.42±0.098^a	2.13±0.15^b	1.47±0.13 ^{e}	1.58±0.14^e

All data are presented as mean ± standard error. Within each row, means with different superscript letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by post hoc (Tukey) test.

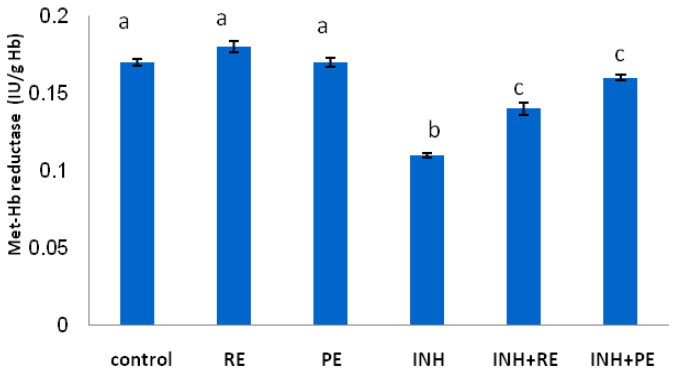


Figure 2. shows blood met-hemoglobin reductase activity of control and treated rats' groups; groups with different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by post hoc (Tukey) test.

Moreover, treatment of rats with the aqueous extract of either rosemary or parsley recorded a Hb-auto-oxidation rate and RBCs hemolysis percentage similar to that of control group, while animals those treated with INH showed significant raise in both Hb-auto-oxidation rate and RBCs hemolysis percentage in compare to normal group. Fortunately, animals groups those were treated with ether rosemary or parsley extracts in combination with INH recorded significant decreases in both Hb-auto-oxidation rates and RBCs hemolysis percentage in compare to INH-treated group (Figure 3 and 4).

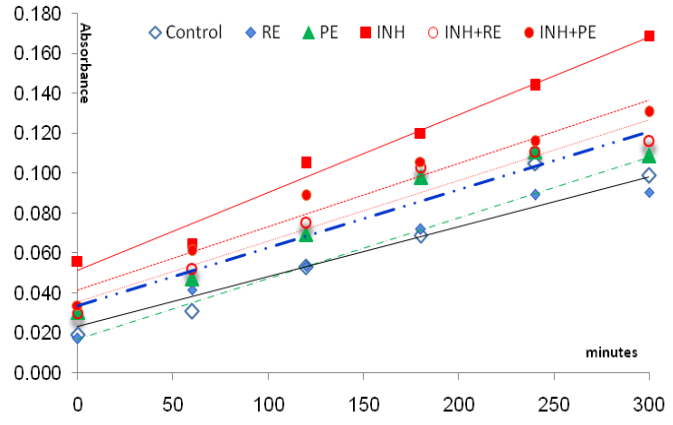


Figure 3. shows auto-oxidation rate of oxy-hemoglobin (HbO_2) of male rats of control and treated animal groups.

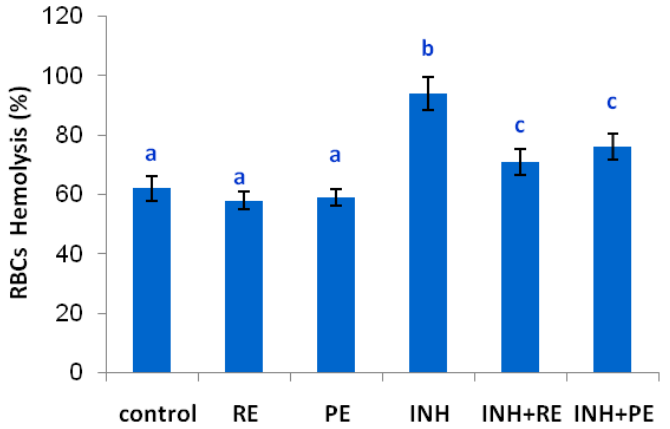


Figure 4. Shows the percentage of hemolysis (under stress of Mercury chloride) of RBCs of control and treated rats' groups.

Discussion

Tuberculosis is a leading public health problem worldwide, particularly in developing countries. There is no suitable drug for treating hematological disorder caused by INH during treatment of tuberculosis patients. In this regard, herbs are implicated as potential protective agents; therefore, the present study attempts to investigate the hematoprotective and antioxidant potential of *Rosmorinus officinalis* and *Petroselinum crispum* in a trial to enhance the oxygen transport and delivery consequently improving the drug efficacy and the body tolerance in tuberculosis patients.

The obtained data of this study monitored that the extracts of either rosemary or parsley performed no adverse effects on RBCs count or hemolysis, hemoglobin, hematocrite, blood indices (MCV, MCH & MCHC), platelets and leucocytes values. This result is in accordance with the reports of Awe & Banjoko [34] and AL-Awaida *et al.* [35]. Also, rosemary and parsley extracts didn't disturb the functional Hb-derivative (Hb-O_2) or the non-functional Hb-derivatives (met-Hb, Hb-Co and Hb-S) percentages, Hb-O_2 auto-oxidation rate, and met-Hb reductase activity; these findings mostly are in agreement with findings of Abdel-Wahhab *et al.* [35]. A safe effect of both extracts on the hematopoietic system can be concluded her.

Controversially, the present study indicated that oral administration with Isoniazid® resulted in occurrence of physiological or hypochromic anemia which monitored from the significant decrease in hemoglobin content,

RBCs count (erythrocytopenia), hematocrite value, MCH, and MCHC; also, thrombocytopenia (decrease in platelets count) and leucopenia (reduction of TLC) were recorded in compare to control group. These data are in accordance with the findings of Badar *et al.* [37], Abdel-Ghaffar *et al.* [38] who reported that chronic application of Isoniazid® induces a reduction in the number of platelets, RBCs and leukocytes through the induced-oxidative stress, which might affect their life expectancy, induce an apoptosis and thereby ultimately reduce the number of these cells in the blood. Also, Abd El Reheem and Zaahkeuk [39] reported that rats orally ingested toxic substances showed a decrease in hemoglobin content; they and attributed this result to one or more mechanisms; the disturbance in iron metabolism including absorption, transport and cellular uptake which led to inhibition of hemoglobin synthesis, as well as reduced erythrogenesis; and/or alternation in the activity of enzymes responsible for heme synthesis, consequently affect the production RBCs and Hb.

Yakup *et al.* [40] suggested that anemia is a well-known side-effect occurs as depletion in RBCs number and maturation as a consequence to INH which causes significant disturbances in hematological parameters (in humans and rats). Moreover, the significant decrease in the activity of met-Hb reductase [the enzyme responsible for reduction of ferric or met-Hb to ferrous or functional Hb-O_2] went in line with reduction in Hb-O_2 fraction and associated with the significant increase in the non-functional hemoglobin fractions (met-Hb, Hb-CO & Hb-S) pointing to a kind of functional anemia in addition to the clinical or physiological one. All of these findings evidencing a weakness in the oxygen battery that supply the tissue demand (tissue hypoxia and/or functional anemia). These results are in agreement with the report of Abdel-Wahhab *et al.* [41] and Kehinde & Adaramoye [42].

Several activities of the antioxidants are mediated by inhibition of reactive oxygen species (ROS) which are generated during the oxidative burst; thus, the usefulness of antioxidants in protecting cellular components against oxidative stress is well established [43]. In most cells, mitochondria are major source of ROS [44]; despite their lack of mitochondria, ROS are continuously produced in the erythrocytes due to the high O_2 tension in arterial blood as well as from their abundant heme-iron content [45].

Anado *et al.* [46] and Xu *et al.* [47] suggesting that the used herbal extracts may stimulate the activity of the bone marrow stem cells, consequently strengthen the erythropoiesis (oxygen-supply agents), systemic and particularly immune cellular defenses of the organism

Direct exposure to molecular oxygen and circulating components in the blood and the loss of the *de novo* synthesizing capacity of new enzyme molecules during maturation put the erythrocytes at high risk of damage by superoxide anion ($O^{\cdot-}$ and H_2O_2) molecules; these reactive molecules are involved in lipid peroxidation [48, 49], oxidation of thiol groups of enzymes [50] and the oxidative degradation and denaturation of Hb [51]. The denaturized hemoglobin (met-Hb) precipitates and covalently binds to the interior erythrocytes membrane, thus forming Heinz bodies; this process distorts the cell membrane, resulting in increased erythrocytes fragility and hemolysis [52]; that is why the RBCs hemolysis and non-functional Hb derivative (met-Hb) percentages were increased in INH-treated animals.

In addition, hemoglobin contains redox-active transition metal iron that makes them susceptible to causing oxidative damage. Although the structure of the globin chain allows heme to bind oxygen with minimal oxidation of ferrous to ferric iron, auto-oxidation is not entirely prevented; low concentrations (less than 2% of circulatory Hb) of methemoglobin are normally present *in vivo*. Met-hemoglobin can then react with the peroxides formed during the auto-oxidation process itself or elsewhere, Isoniazid® metabolites, in the protein's vicinity; both the globin-bound radical and ferryl heme iron can cause tissue damage, by initiating lipid peroxidation reactions [53-57].

Moreover, when Isoniazid®-treated rats orally supplied with antioxidants, such as herbal extracts, they performed marked improvement in blood picture, this may be attributed to the antioxidant potential (directly by scavenging free radicals or indirectly by improving the enzymatic or non-enzymatic antioxidant systems of the rats tissues) that protects erythrocytes from the oxidative damages induced by toxic metabolites of INH. Treatment with rosemary or parsley extract could decrease lipid peroxidation in red blood cells that was elevated by Isoniazid®, consequently reduce the exhaustion of non-enzymatic antioxidant agents or inhibition of the antioxidant enzymes. Rosemary and parsley also improved hemoglobin function since they could elevate total Hb and the functional Hb (Hb-O_2) levels and decrease the non-functional form (met-Hb). These data further confirm the antioxidant potential of rosemary and parsley [58]; other author interpreted these improvements to the fact that rosemary and parsley contain a number of bioactive compounds which are generally believed to be the active constituents responsible for their antioxidant activity. These constituents that responsible for the antioxidant activity of the tested materials are including flavonoids, tannins, sterols phenolic diterpenes, phenolic acids and/or triterpenes [59].

Conclusion

Rosemary and parsley aqueous extracts showed an ameliorative-antioxidant benefits as well as hematoprotective effects; the efficiency of rosemary extract was higher than that of parsley; this performance

could be attributed to the antioxidant activity of their major constituents.

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