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The Effect of Alcoholic Extract of Pomegranate Peel on the level of Erythropoietin and some of the Blood Properties in Albino Male Rats Treated with Erythromycin

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Abstract--- *The study was conducted to evaluate the possible protective effects of alcoholic extract ofthe pomegranate peels against the Erythromycin antibiotic. The study was performed in the animal house of the Department of Biology, College of Education for Girls, University of Kufa, by following (20) Albino male rats from the (Sprague Dawley) strain, which was divided into four groups and each group consists of five males, that weighted between (200-250)g, and the first group was the control group that was orally given physiological solution(0.9%), while the second group was administrated the antibiotic Erythromycin at a concentration of (500) mg / Kg orally, and the third group, it was treated with alcoholic extract of the pomegranate peels with a concentration of (400) mg / kg + Erythromycin with a concentration of (500) mg / kg orallytoo. The last group was orallysubmitted to the alcoholic extract of the pomegranate peels with a concentration of (400) mg / kg, and all the treatments were conducted twice a day, in the morning at (eight in the morning) and the second one at (eight in the evening) every day for fourteen consecutive days. The study included some physiological criteria for the blood, which comprises the numbers of the red blood corpuscles, hemoglobin level, packed cellvolume, platelet count, and total differential count of white blood cells. As for the biochemical study, it included estimating the level of the erythropoietin hormonein the serum. The results of this study showed a significant increase (P< 0.01) in the number of erythrocytes, the level of hemoglobin, and packed cell volume in the group that treated with alcoholic extract of pomegranate peels in compared to the control group and other experimental groups. No notable difference (P> 0.01) was shown in the other criteria that included in the current study, on the contrary, a remarkable decrease (P< 0.01) was observed in red blood corpuscle numbers, hemoglobin level, packed cell volume, platelet count, and percentages (P< 0.05) of lymphocytes, monocytes, acidophils, basophils and erythropoietin hormone level in the group that were treated with Erythromycin, otherwise a significant increase (P <0.01) was noticed in the total count of white blood cells, and the percentage of neutrophils in the group that subjected to Erythromycin when compared with the control group and other groups of study. Moreover, no significant differences(P> 0.01) were obtained between the other study groups. As for the group that was treated with alcoholic extract of the pomegranate peels + Erythromycin, no observable changes(P> 0.01) were shown in comparable to the other groups of experiment. It was concluded from this study that the alcoholic extract of the pomegranate peels has a preventive effect against the toxic impacts that associated with the Erythromycin antibiotic, which may have been caused by its potent anti-oxidant chemical contents.*

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I. INTRODUCTION

Recently it was recognized all over the world the importance of traditional medical practices, as there must be a systematic practical importance of assessing and knowing the efficacy of the drugs or medicines that are of herbal origin in order to benefit from the active substances and compounds in which they are found [1], and many natural oxidative antigens have been discovered that can be classified as a secondary receptor in plants, examples include flavonoids, turbines, and carotene,and eating foods that contain this form of compounds have an importance in protecting against many diseases [2]. It is known that compounds with large rings have great potential for use widely in treating various diseases, and therefore they manufacture very important molecules that are used most drugs with large rings to treat mostly infectious diseases, but they are also used in the treatment of cancer and bacterial diseases, [3] because they possess significant anti-infective and immunity properties [4], as well as tuberculosis, antifungal, parasites, malaria, and viruses, in addition to its anti-cancer properties [5].The compounds known as macrocyclic macrolides are natural compounds produced by the types of Streptomyces which are the most common class of antibiotics and it also belongs to the category of macrolides or periodic peptides, and the first macrolides which consists of 14-members, that is Erythromycin A, which has been used clinically since 1952, and it is active against the positive bacteria of the gram stain and some negative microorganisms of the gram stain, furthermore, it is used in the treatment of infections of the respiratory system, digestive system, genitals and soft tissue infections [6].Moreover, to improve acid stability and oral bioavailability of Erythromycin A, natural or semi-synthetic macrolides of the first generation are used, such as spiramycin [7].Erythromycin is a macrolide group antibiotic that is recommended as a primary treatment to prevent widespread infection caused by fungi in AIDS patients and to treat pulmonary infections in HIV-infected patients [8].Although it was considered a safe drug with mild symptoms in the digestive system, as well as a rash [9]. The high doses of Erythromycin used to treat the Mycobacterium cause tinnitus, dizziness, hearing loss, and the risks associated with death are hepatotoxicity and aural toxicity, which are from the negative reactions that appear when using macrolide antibiotics, especially Erythromycin[10].Pomegranate is an important source of bioactive compounds. It has been used in the herbal medicine for centuries, and it has been proven that pomegranate juice is rich in anti-oxidant activity and it is effective in preventing atherosclerosis, in a previous study, it was found that pomegranate peels had a higher antioxidant activity than pulp and pulp shells compared with the seeds of 28 types of fruit that are usually consumed in China as determined by the FRAP standards (ferric reducing ability of plasma) which means marginal anti-oxidant capacity, the results also showed that pomegranate peel extract has a markedly higher antioxidant activity than pulp extract against the negative oxide foxide, hydroxyl and peroxyl, as well as inhibition of oxidation of low-density lipoprotein(11). It is interesting that pomegranate peels were used in ancient times in the Middle East as a colorant for textiles because of the high tannin and phenol content in them [12], and the methanol extracted from the pomegranate peel shows a high capacity as antioxidants [13]. In addition, pomegranate peels are characterized by an internal network of membranes that make up approximately 30-26% of the total fruit weight and are characterized by large amounts of phenolic compounds including flavonoids (anthocyanins, catechins and other flavonoids)and biodegradable tannins [14].The therapeutic properties of pomegranate peels have been identified in various regions and cultures,in

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Egyptian culture, pomegranate peels have been known in treating many common diseases, such as infections, diarrhea, intestinal worms, coughs and infertility [15]. Many studies have shown important potential of the pomegranate peels as antibacterial and worms, as well as its protective and curative role in gastrointestinal infections and cancer prevention [16].

II. MATERIAL AND METHODS

1. Preparation of the Laboratory Animals

A group of male Albino rats, (20) of the Sprague Dawleystrain were used in the study, they weighted between (200-250)g and less than three months age (which means, they did not reach to the sexual maturity). Animals were obtained from veterinary medicine, they entered the animal house of the College of Education for Girls / University of Kufa, on the 1/9/2019,and were treated with great care under appropriate laboratory conditions of constant lighting (13 hours of light / 11 hours of darkness),good ventilation and air-conditioned room in which the temperature ranged Between (22-28) Cº, sawdust was placed in the cage floor, and the cage floor was changed twice a week to maintain cleanliness, as well as, the rats were given water and animal feed intended for them that was rich in protein until they were three months old, then they were subjected to the experiment and implementation of work.

2. Pomegranate Peels

Pomegranate peels that used in the current study were brought from local markets, diagnosed and classified by the specialized plant classification professors.

3. Preparation of Alcoholic Extract of Pomegranate Peels

The preparation of alcoholic extract of pomegranate peels was according to the method of [17]

4. Preparation of the Erythromycin Antibiotic

The antibiotic at dose (500 mg) brought from the (safety first pharmacy) in Babel / Al-Hilla Center, the manufacturer company is (Marksans Pharma Ltd). In this study, hard capsules of Erythromycin used, then they were grinded with a mixture until they became flour,after that dissolved with physiological solution and gave to the experimental rats according to the body weight [18].

5. Experimental Groups of the Study

The first group, which included 5 rats dosed with physiological solution for a period of fourteen days without interruption and twice a day, which is the control group. The second group contained 5 rats orally administrated with the twice daily prepared Erythromycin for fourteen days and also without interruption, at a concentration of (500) mg / kg. The third group comprised 5 rats were orally given the alcoholic extract of pomegranate peels with a concentration of (400) mg / kg, and after an hour it was orally treated with Erythromycin at a concentration of (500) mg / kg twice a day for fourteen days without interruption.

The fourth group, also included 5 rats orally submitted to the alcoholic extract of pomegranate peels prepared in advance for twice day at a concentration of (400) mg / kg for fourteen days without interruption.

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6. Sacrificing of the Laboratory Animals and Collection of Blood Samples

After the dose period end for all groups, the rats were anesthetized with (Diethyl ether), then the blood was drawn from the heart directly before the dissection with a (heart puncture) to obtain (3) ml of the blood, (1) ml of it has been saved in a tube containing an anticoagulant(EDTA) to study the physiological parameters of the blood, while the remaining (2) ml kept in a Gel tubeto evaluate the level of the erythropoietin hormone in the serum.

7. Study of Physiological Standards of the Blood

Some physiological parameters of the blood were estimated by using the (Hematology analyzer Ruby device), which was made by the German company Abbott.

8. Estimation of the Level of Erythropoietin in the Serum

To estimate the hormone level, the method of[19] followed, and the chemical kits, as well as reagents that used in this experiment were purchased from the Kilal company(ILP-international GMBH-Germany).

9. The Statistical Analysis of the Study

The statistical analysis of the data of the current study was performed by using the SPSS program version 24, then the descriptive analysis used to extract the mean and standard error (Mean ± SE), while the study groups were compared with each other's by the ANOVA test (Contrast analysis test) and the value of the least significant difference LSD, under probability level 0.01 [20].

III. RESULTS

The results of the current study revealed a significant increment ($P< 0.01$) in the number of erythrocytes, the level of hemoglobin and the packed cell volume in the group that was administrated with the alcoholic extract of pomegranate peels in comparable to the control group and other experimental groups, and no significant difference ($P >0.01$).

In the other criteria included in the present study, in contrast, a noticeable decrement ($P< 0.01$) was observed in red blood corpuscles, hemoglobin level, packed cell volume, platelet count, and percentages ($P< 0.05$) of lymphocytes, monocytes, acidophils, basophils and erythropoietin hormone level in the group that was treated with Erythromycin, otherwise a significant increase ($P< 0.01$) was observed in the total count of white blood cells, and the percentage of neutrophils in the group subjected to the antibiotic when compared with the control group and other groups of study.

On the other hand, no significant alterations ($P> 0.01$) were noted between the other study groups.

As for the group that treated with alcoholic extract of pomegranate peels + Erythromycin, no significant changes ($P< 0.01$) were shown when compared with the other experimental groups. As in the tables (1,2,3, and 4) respectively.

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Table 1: Effect of Treatment with Alcoholic Extract of Pomegranate Peels and Erythromycin on Erythrocyte Numbers, Hemoglobin Level, and Packed Cell Volume

Groups of study	Mean ± standard error			
	Number of samples	Hemoglobin level g / dl	Erythrocyte numbers / mm 10^6	Packed cells volume%
Control group	5	14.602 ±0.12 a	6.7304±0.12 a	41.02± 0.15 a
Erythromycin group(500)mg /k g	5	11.642±0.26 b	4.0996±0.18 b	34.52± 1.11 b
Pomegranate peels alcoholic extract group(400) mg / kg	5	16.08±0.23 c	7.1384±0.23 c	43.78± 0.79 c
Group of pomegranate peels alcoholicextract (400)+Erythromycin(500)mg/kg	5	14.66±0.30 a	6.713±0.199 a	41.08± 0.21 a
LSD		0.716	0.558	2.19

- **Symmetric letters mean that there were no significant differences at (P< 0.01) level between groups.**
- **Letters that are not identical mean significant differences at (P< 0.01) level.**

Table 2: Effect of Treatment with Alcoholic Extract of Pomegranate Peels and Erythromycin on the Number of Platelets and Total Count of White Blood Cells

Groups of study	Mean ± standard error		
	Number of samples	Number of platelets / mm^3 x 10^3	Total count of white blood cell cells / mm^3
Control group	5	430.6±4.65 a	4739.4± 237 a
Erythromycin group (500) mg / kg	5	338.2 ± 19.05 b	9159.6± 481 b
Pomegranate peels alcoholic extract group(400) mg / kg	5	438.6± 4.06 a	4837.4 ± 154.8 a
Group of pomegranate peels alcoholic extract (400)+Erythromycin (500)mg/kg	5	437.8± 3.92 a	4835.4 ±149.5 a
LSD		30.60	866.3

- **Symmetric letters mean that there were no significant differences at (P< 0.01) level between groups.**
- **Letters that are not identical mean significant differences at (P< 0.01) level.**

Table 3: The Effect of Treatment with Alcoholic Extract of Pomegranate Peels and Erythromycin on the Differential Count of White Blood Cells

Groups of study	Mean ± standard error					
	Number of samples	Neutrophil%	Lymphocyte%	Monocyte%	Acidophil%	Basophil%
Control group	5	70.242 ± 0.61 a	23.98± 0.33 a	3.964± 0.29 a	1.802± 0.11 a	0.022± 0.007 a
Erythromycin group(500)mg/ kg	5	77.762 ± 0.88 b	20.382± 0.30 b	1.466 ± 0.18 b	0.408± 0.06 b	00±00 b
Pomegranate peels alcoholic extract group(400) mg/ kg	5	70.146 ± 0.63 a	24.196± 0.25 a	3.892± 0.21 a	1.77± 0.08 a	0.012±0.003ab
Group of pomegranate peels alcoholic extract (400)+Erythromycin (500)mg/kg	5	70.198 ± 0.75 a	24.142± 0.14 a	3.884± 0.16 a	1.792± 0.07 a	0.008±0.002ab
LSD		2.188	0.81	0.657	0.256	0.0127

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- **Symmetric letters mean that there were no significant differences at the (P> 0.01) level between the groups**
- **Letters that are not identical mean significant differences at (P< 0.01) level**

Table 4: Effect of Treatment with Alcoholic Extract of Pomegranate Peels and Erythromycin on the Level of Erythropoietin in Serum

Groups of study	**Mean ± standard error**	
	Number of samples	**Erythropoietin mmol/ml**
Control group	**5**	**14.804±0.60a**
Erythromycin group (500) mg / kg	**5**	**5.92± 0.51 b**
Pomegranate peels alcoholic extract group(400) mg/ kg	**5**	**16.326± 0.80 a**
Group of pomegranate peels alcoholic extract (400)+Erythromycin (500)mg/kg	**5**	**14.806± 0.96 a**
LSD		**3.07**

- **Symmetric letters mean that there were no significant differences at the (P< 0.01) level between the groups**
- **Letters that are not identical mean significant differences at (P< 0.01) level.**

IV. DISCUSSION

1. Effect of Treatment with Alcoholic Extract of Pomegranate Peels and Erythromycin on the Erythrocyte Numbers, Level of Hemoglobin, and Packed Cell Volume

The dosage with alcoholic extract of the pomegranate peels caused a significant raise in the red blood corpuscles count, and the level of hemoglobin, as well as the packed cell volume, which came in agreement with some studies [21], but it differed with others. [22]. It is possible that the result is explained by the fact that the extract contains many active chemical components, especially vitamin C [23] and flavonoids [24], different types of tannins [25] and other biologically active substances as demonstrated by the preliminary examination documented in the current study, which may have a positive effect on stimulating bone marrow tissues to increase the production of red blood corpuscles, and hence notably increasing its numbers in the bloodstream, as well as their hemoglobin content.. Besides, pomegranate peels also contain many antioxidants that work on scavenging the harmful free radicals and get rid of its cellulartoxic effects[26], which has led to maintaining selective permeability of the red blood corpuscle membranes and keeping their nucleic, as well as cytoplasmic components, in addition improving their efficiency in transporting oxygen to the body cells. Moreover, the powerful anti-oxidative compounds of pomegranate peels, through their supporting to the enzymatic and non-enzymatic antioxidant systems, may have stimulated a remarkable increase in the number of red blood corpuscles in the blood, as well as their hemoglobin levels and packed cell volume. In addition to the above, the alcoholic extract of pomegranate peels may contain (Iron pyridoxine) and other basic materials which may have a potent effect on increasing the level of hemoglobin in the red blood corpuscles, or they, along with other components with high biological effectiveness, have worked synergistically to improve the absorption of iron from the gut and thus increased numbers of erythrocytes, their

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hemoglobin content and the packed cell volume accordingly. On the contrary to the above, the group that was orally given Erythromycin showed a significant decline in these criteria, in agreement with some studies[27]. This may be explained by the fact that Erythromycin may activate the process of peroxidation of the phospholipids that present in the erythrocyte membranes,and subsequent increase in the production of various reactive oxygen species (ROS) due to oxidative stress, which may cause the loss of the selective permeability of membranes for those corpuscles and the exit of their cytoplasmic contents outside, thus reducing their numbers substantially in the bloodstream, as well as their hemoglobin content and packed cells volume. Or perhaps the antibiotic changed the form of erythrocytes due to oxidative stress and the subsequence inflammatory processes stimulated by that antibiotic from the double-sided concave to the prolonged or twisted or other forms that are much less efficient to transport oxygen to the tissues of the body,and therefore their numbers and content of hemoglobin, as well as the packed cell volume have recorded a significant decrease.Furthermore, the reason may be attribute to the ability of Erythromycin to associate with erythrocytes, which indicates a slight absorption of the antibiotic by erythrocytes, in addition to the interference between this type of the antibiotic and the cell membrane, as confirmed by some studies [28], so the count of red blood corpuscles significantly declined. In addition to the foregoing, the observable decrease in the level of the hormone erythropoietin in laboratory animals that treated with Erythromycin may be caused inhibition to the production of erythrocytes from the bone marrow, as well as reducing the level of hemoglobin in them, and the packed cell volume because it plays a substantial role in stimulating their production from the bone marrow tissues [29].With regard to the group that was orally submitted to the alcoholic extract of the pomegranate peels + Erythromycin, it did not indicate any significant change in these vital parameters, which may attribute to the preventive efficiency of its medically effective components of tannins and phenols [11].

2. The Effect of Treatment with Alcoholic Extract of Pomegranate Peels and Erythromycin on the Number of Platelets

The statistical analysis of findings of the current study did not show notable differences in the number of blood platelets in the group that was treated with the alcoholic extract of pomegranate peels, but it did not agree with some studies [22]. This possibly because its potent anti-oxidative constituents of tannins [30], phenolic, flavonoids, and saponins [31] and other contents found in pomegranate peel extract did not negatively or positively affect the effectiveness of megakaryocytes present in the marrow bone tissues and responsible for the production of platelets, and thus the rates of its mitotic divisions were not significantly affected, which led to the result that their numbers did not alter remarkably in the blood.

Regarding the group was orally subjected to the Erythromycin, it revealed a decrease in the number of platelets when compared with other experimental groups, which was agreed with many studies [32].This can be explained by the high rates of oxidative stress and the increment in the production of deteriorative free radical (ROS) due to the peroxidation of phospholipids in the blood platelet membranes, which caused the possibility of breaking them in the bloodstream and consequently reducing their numbers significantly, as the membrane of these platelets consists of many phospholipids,glycoproteins and integrins that play a vital role in the adhesion and functional performance of platelets, as well as they contain three types of granules, which are dense and lysosomal, alpha granules and other structures inside the platelets or in their membranes are very important to the internal balance of the body [33], so

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perhaps the antibiotic used in this study has negatively affected the cellular structures of these components, and consequently, their numbers were affected and decreased substantially in the bloodstream. Or that the increased production of reactive oxygen species under the negative influence of the antibiotic has stimulated platelets to form a blood clot, which led to raise the rates of cellular insertion of the calcium ion, as well as stimulate the process of phosphorylation of the tyrosine protein as a type of response to the formation of the so-called thrombin and thus the platelet adhesion to each other and to significantly decrease its number in the blood.

Or perhaps the antibiotic has caused a rise in the rates of bio-synthesis of thromboxane A2 in the blood platelets, which directly helped to encourage their accumulation and adhesion to each other and to reduce their numbers markedly in the blood, or it inhibited the mitotic divisions of megakaryocytes in bone marrow tissues directly, which reflected negatively on their numbers in the blood.

With regard to the group that was given the extract + Erythromycin, it did not show a significant change in the number of platelets, which may be explained by the efficiency of its preservative and therapeutic components [34], in maintaining the cellular membranes of those platelets intact and that the cytoplasmic contents, as well as the nucleus inside the cell remain undamaged, hence their numbers didn't noticeably altered within the bloodstream.

3. The Effect of Treatment with Alcoholic Extract of Pomegranate Peels and Erythromycin on the Total and Differential Count of White Blood Cells

From the data of the statistical analysis, the group that was administrated with pomegranate peels extract did not reveal a significant change in the total number of white blood cells, which came in agreement with some studies [22].This may explain that pomegranate peels extract contains numerous potent medicinal substances such as saponins, phenols, flavonoids, alkaloids, mineral salts and vitamins [35] which may have worked together to protect various body tissues particularly the more sensitive organs as gut, liver, kidneys from acute and chronic infections and, thus not affecting the total number of the white blood cells whose number increases significantly when the body's cells are exposed to diseases and to any type of inflammation [36].In contrast to the foregoing, the total number of white blood cells in the group treated with Erythromycin showed an observable raise in compared to other experimental groups, and this result was consistent with some studies [37].The finding possibly attribute to the notable increase in the percentage of neutrophils that were documented during the current study among the rats that were given Erythromycin because it constitutes 70% of the total white blood cells, so its significant raise in the blood would cause an increase in the total number of the white blood cells in the bloodstream [38].

As for the group that was orally submitted to the alcoholic extract of pomegranate peels + Erythromycin, it did not indicate a significant change in the total number of white blood cells, perhaps this is due to the preventively active components of the extract against the side effects associated with the use of the antibiotic from oxidative stress and subsequent generation of the various reactive oxygen species,and thus it provided a complete protection to all tissues of the body, in particular the liver and kidneys, from the inflammatory effects of Erythromycin, which reflected positively by not markedly changing the total number of white blood cells. In relation to the count of white blood cells in the group treated with the alcoholic extract of pomegranate peels, there was no significant difference compared with the other groups of study, which was in agreement with some studies [22]. Perhaps the result is

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explained by the biological components of the alcoholic extract of pomegranate peels with preventive and curative properties against various diseases, tumors, infections [39], hepatotoxicity [40] and renal toxicity [41], which confirms that the continuation of the dose with this extract for a period of (14) days did not conflict with the physiological functions for any type of white blood cells, and therefore their percentages in the blood didn't significantly indicate a negative or positive change.

Contrary to the above, the percentage of neutrophils in the blood has witnessed a significant increase in the group that was orally given the antibiotic Erythromycin and this was in agreement with some studies [42], but it differed with others [43]. The result may be attributed to the pathological effects caused by this type of antibiotic due to the induction of lipid oxidation processes in the cell membranes and the consequent increased production of detrimental free radical such as hydrogen peroxide and negative hydroxyl radicals that are associated with sensitive biomolecules, especially the nitrogenous bases of DNA, and the carbohydrate, protein and fatty molecules that cause imbalance to the anti-oxidative (enzymatic and non-enzymatic) systems and disruption of mitochondrial functions, which would stimulate a rise in neutrophils as they are the first defensive line because of their ability to attract towards the affected place(Positive chemotaxis) and its amoeboid movement within the narrow blood vessels, as well as its high-phagocytic properties, in addition to contain powerful enzymes capable of digesting proteins [36]. In contrast to the above, the percentage of lymphocytes decreased significantly in the group that was given the antibiotic and it differed with some studies [32].

It may explain that Erythromycin has suppressed the immune system and the mitotic divisions of this type of cell, or has suffered programmed death by the effect of continuous production of toxic free radicals.

The finding also attribute to the effect of the oxidative stress state that activated by the antibiotic treatment, which could have caused an increase in the secretion of glucocorticoids, especially the hormone cortisol from the adrenal gland, which actually appears to inhibit the releasing of these cells from the bone marrow tissues, which causes a significant decrease in their percentages in the bloodstream [36].

As for the monocytes, they also revealed a significant decline in their percentages in the male group that treated with Erythromycin, and the result is compatible with some studies [32]. This may be because of the negative effect of the peroxidation process and the subsequent increase in the rates of generation of reactive oxygen species with a destructive action to bone marrow tissues, so the precursor cells for this type of the cells decreased, and it was reflected negatively by a significant decline in the percentage of monocyte in the bloodstream, as it represents the second defense line of the body has higher phagocytic properties than neutrophils and replaces them when inflammation becomes chronic [38]. A significant decrease observed in the percentage of acidophils in the experimental group that treated with Erythromycin, and this was came along with some studies [32]. The finding possibly explain to the increased levels of oxidative stress hormones, particularly cortisol [36], due to the detrimental impact of the antibiotic that used in this study and the destruction effects of these hormones cause the removal for this type of blood cells from the bloodstream.

The current study has also found a significant decrease in the percentage of basophils, this probably due to the continuous treatment for 14-days with Erythromycin which may cause severe stress for all body cells, and the

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consequent increase in the levels of bio-synthesis of glucocorticoids, thus increase their levels in the blood and decrease the percentages of basophils accordingly. Concerning the group that administrated with alcoholic extract of pomegranate peels + Erythromycin, no significant difference was shown in the number of white blood cells. Perhaps the reason is that the pomegranate peel is a good source of biologically active compounds such as catechins, ellagitanins, epicaticin, routines, and many others, which are responsible for numerous vital activities such as antimicrobial and antioxidant [44].

4. The Effect of Alcoholic Extract of Pomegranate Peels and Erythromycin on the Level of the Hormone Erythropoietin

The present study did not show a significant difference in the level of the erythropoietin in the group treated with the alcoholic extract of pomegranate peels, which came in agreement with some studies [45], but it differed with others [22].This may be explained to the physiological efficiency of the extract components in protecting the hepatic [13] and renal [41] tissues from various infections and diseases [39], as well as ameliorating their functional performance, as 90% of the hormone is secreted from the per tubular interstitial cells and the remainder (10%) of liver tissues and other body organs [38]. Contrary to the above, the results of the study showed a significant decrease in the level of the hormone erythropoietin in the group that orally given Erythromycin. This probably due toa fibrosis of peritubular interstitial cells that produced erythropoietin, and thus lower the rates of production of hormone, as demonstrated by some studies [46]. Moreover, the decrement in the hormone level may be attributed to the partial or perhaps complete damage to the nerves contained in the renal tissues due to the free radicals whose production rates were stimulated because the side effects of the antibiotic used in this study, which caused a significant reduction in the level of erythropoietin in the blood. In addition to what have been mentioned above, the reduction of erythropoietin level may be explained by the possibility of a failure in the mechanism related to sensitivity to the oxygen level by the peritubular interstitial cells in the renal tissues, or perhaps because of the production of a certain type of inflammatory cytokines that generate many signals play essential role in the regulating of immune system, which in turn works to suppress the molecular manufacturing of this hormone and its inhibition, as proven by some studies [46].With regard to the group that was submitted to the alcoholic extract of pomegranate peels + Erythromycin, it did not indicate a significant change in the level of the hormone erythropoietin, and it is likely that this is because the effective chemical contents with medicinal properties, especially the antioxidants such as vitamin C [12], which are used in the prevention of hepatotoxicity [40, 44],nephrotoxicity [48],inflammatory conditions and various diseases [47,49],as well as cancer [14, 46], hence there was no noticeable change in the level of hormone in the blood.

V. CONCLUSION

The alcoholic extract of pomegranate peels showed a marked improvement in some physiological parameters of the blood, due to the preventive and anti-oxidative potentialities of its chemical constituents, furthermore it protected the sensitive tissues of kidney from the toxic impacts of Erythromycin that was used in the current study, therefore the levels of erythropoietin did not reveal a marked difference, so the study recommend to use the extract of pomegranate peels as a dietary complement or may be a natural remedy.

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REFERENCES

[1] Muthuraman, A.; Sood, S. and Single, S.K. (2011). The anti inflammatory potential of phenolic compounds from *Emblicaofficinalis* L.in rat. *Infalmmo. Pharmaco.,* 19(6): 327-334.

[2] Seif, R.A. (2016). Physiological change due to hepatotoxicity and the protective role of some medicinal plants. Beni-suef Un. *J. Basic. Applied.Sci.,* 5(2):134-146.

[3] Labro, M.T.(1998). Anti-inflammatory activity of macrolides: A new therapeutic potential. *J. Antimicrob. Chemother.,* 41: 37–46.

[4] Marjanovic´, N.; Bosnar, M.; Michielin, F.; Willé, D.R.; Anic´-Milic´, T.; ˇCulic´, O.; Popovic´-Grle, S.; Bogdan, M.; Parnham, M.J. and Erakovic´ Haber, V.(2011). Macrolide antibiotics broadly and distinctively inhibit cytokine and chemokine production by COPD sputum cells in vitro. *Pharmacol. Res.,* 2(63):389–397

[5] Bukvic´ Krajacˇic´, M.; Peric´, M.; Smith, K.S.; Ivezic´ Schönfeld, Z.;Žiher, D.; Fajdetic´, A.; Kujundžic´, N.; Schönfeld, W.; Landek, G.andPadovan, J.(2011). Synthesis, structure-activity relationship, and antimalarial activity of ureas and thioureas of 15-membered azalides. *J. Med. Chem.,* 54: 3595–3605.

[6] Omura, S. and Shiomi, K. (2007). Discovery, chemistry, and chemical biology of microbial products. *Pure Appl. Chem.,* 79:581–591

[7] Kaufman, H.E. (1961). Spiramycin. Arch. Ophthalmol., 66: 609–610

[8] Kovacs, J.A. and Masur, H. (2000). Prophylarix against opportunistic infections in patients with human immunodeficiency virus infection. *N Engl. J. Med.,* 342: 1416-29.

[9] Le Clerq, R. and Courvalin, P. (1991). Intrinsic and unusual resistance to macrolide, lincosamide, and streptogramin antibiotics in bacteria. *Antimicrob. Agents. Chemother.,* 35:1273-6

[10] Jelic, D. and Antolovic, R. (2016). From Erythromycin to Azithromycin and New Potential Ribosome-Binding Antimicrobials. *J. Antibiotics.,* 3-13.

[11] Li, Y.; Guo, C.; Yang, J.; Weu, J.; Xu, j. and Cheng, S.(2006). Evaluation of antioxidant properties of Pomegranate peel extractin comparison with pomegranate pulp extractb. Food. Chem., 96., 2.

[12] Singh, R.P.; Murthy, K.N.C. and Jayaprakasha, G.K.(2002).Studies on the antioxidant activity of pomegranate peel and seeds extract using in vitro models. *J. Agri.. Food. Chem.,* 50: 81-86.

[13] Murthy, K.N.CH.; Jayaprakasha, G.K. and Singh, R.P. (2002). Studies on antioxidant of pomegranate (*Punicagranatum*) peel extract using in vivo models. *J.Agriculture. food.Chem.,* 50 (17): 4791-4795

[14] Afaq, F.; Saleem, M.; Krueger,C.G.; Reed, J.D. and Mukhtar. H.(2005) Anthocyanin-and hydrolysable tannin-rich pomegranate fruit extract modulates MAPK and NF- kappaB pathways and inhibitsskin tumorigenesis in CD-1 mice. *Int. J. Cancer*., 113: 423-433.

[15] Lansky, E.P. and Newman, R.A. (2007). *Punicagranium* (Pomegranate) and its potentialfor prevention and treatment inflammation and cancer. *J. Ethno. Pharmocol*., 109: 177-206.

[16] Al-zoreky, N.S. and Nakahara, K. (2003). Antibacterial activity of extract from some addible plants commonly consumed in Asia. *Int. J. Foof. Icrobiol*., 80: 223-230.

[17] Grand, A.; Woudergem, P.A.; Verportes, R. and Pousset, J.L. (1988). Antiinfectionphytotherapiesoftree-savannahsengl (West- Africa) II-Antimicrobial activity of 33species. *J. Ethnopharmacol.,* 22: 25-31.

[18] Curatolo, W.; Foulds, G. and La Badie, R.(2010).Mechanistic study of the Azithromycin Dosage-Form-*Dependent Food Effect. Pharm. Res.,* 27: 1361-1366.

[19] Kricka, L.J. (1999). Human anti-animal antibody interferences immunological assays. *Clin. Chem.,* 45:942-965.

[20] Morgan, G.A.; Leech, N.A.; Gloecner, G.W. and Barrett, K.C. (2010). SPSS for introductory statistic: use and interpretation. 2nded. Lawrenz Erlbumassociatiates, publishers *Mahwah, New Jersey. London.*

[21] Machado, T.B.; Leal, I.C.; Claudia, A. and Amaral, F. (2002). Antimicrobial Ellagitannin of *Punicagranatum* fruits: *J. Braz. Chem. Soc.,* 13(5).

[22] Hasan, S.M.H.; Abou-Rawash, A.A. and Bekheet, M.S. (2016). Protective role of an aqueous extract of *Punica Granatum* (Pomegranate) peel on lead-induced anemia in rats. Alex. *J. Veterinary. Sci.,* 50(1): 99-108.

[23] Ramadan, A.; El-Badrawy, S.; Abd-el-Ghany, M. and Nagib, R. (2009).Utilization of hydro-alcoholic extracts of peel and rind and juice of pomegranates natural antioxidants in cotton seed oil. *The 5th Arab and 2nd International Annual Scientific Conference. Egypt:* 8-9.

[24] Tehranifar, A.; Zarei, M.; Nemati,Z.; Esfandiyari, B. and Vazifeshenas, M. R.(2010). Investigationof physic-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punicagranatum* L.). *Sci. Hortic..,* 126:180–185.

[25] Wang, R.; Ding, Y.; Liu, R.; Xiang, L. and Du, L. (2010). Pomegranate: Constituents, Bioactivities and Pharmacokinetics. *Global. Sci. Books.,* 4(2):77-87.

[26] Halliwell, B. and Gutteridge, J.M.C. (1999). Free radical in biology and medicine, 3thed. Oxford. Uni. Press., 1-936.

DOI: 10.37200/IJPR/V24I4/PR2020445
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[27] Innocentia, M.N.; Shino, Y.; Miki, M.A.; Keisuke, S.; Kenichi, W.; Xuenan, X. and Noboru, I. (2017). Oral administration of azithromycin ameliorates trypanosomosisin *Trypanosomacongolense*–infected mice. *J. Parasitolo. Res.*, 116(9):2407-2415.

[28] Deuticke, B. (1977). Properties and structural basis of simple diffusion pathways in the erythrocyte membrane. *Rev. Physiol. Biochem. Pharmacol.*, 78: 1–97.

[29] Ifeanyi, O.E. (2015). A Review on Erythropoietin. *Int. J. Adv. Res. Biol.Sci.*, 2(4):35-47.

[30] Reed, J.D (1995).Nutritional toxicology of tannic and related polyphenols in *Forage legumes. J. Anim. Society.*, 73 (5):1516-1528.

[31] Nantitanon, W.; Yotsawimonwat, S. and Okonogi, S. (2010). Factors influencing antioxidant activities and total phenolic content of guava leaf extract. LWT. *Food. Scien. Technoly* 43: 1095 – 1103

[32] Chukwunelo, A.C.; Anosike, A.C.; Nnamonu, E.I.; Ekpo, D.E.; James, P.O. and Okonkwo, T.I. (2019). Evaluation of leukocyte mobilization and platelet aggregatory Effects of ciprofloxacin, lincomycin and erythromycin in Wister albino rat. *Not. Sci. Biol.*, 11(4):345-351.

[33] Bambace, N.M. and Holmes, C.E. (2011). The platelet contribution to cancer progression. *J. of Thromb and Haemost.*, 9: 237-249.

[34] Middleton, E. and Kandaswami, C. (1992). Effects of flavonoids on immune and inflammatory functions. Bioch. *Pharmacol.*43:1167-1179.

[35] Wang, Z.; Pan, Z.; Ma, H. and Atungulu, G.G. (2011).Extract of phenolicsfrom pomegranate peels. *J. Food. Sci. Open.*, 5:17-25.

[36] Guyton, A.G. and Hall, J.E. (2006). Textbook of medical physiology 11th ed. Philadelphia, Pennsylvania, *Elsevier Saunders.* P: 315-359.

[37] Sadeq, O.R. and Abu Mowais, M. (2018). Erythromycin: its glycemic effect in an infectious patients. *Int. Res. J. Pharm.*, 9(9):25-29.

[38] Hoffbrand, A.V.; Moss, P.A.H. and Pettit, J.E. (2006). Essential hematology.5th ed.Blackwell publishing.*London.UK.P:*12-21.

[39] Chidambara, M. K.; Reddy, V. K.; Veigas, J. M.; Murthy, U. D. (2004). Study on wound healing activity of *Punicagranatum* peel. *J. Med. Food.*, 7:256–259.

[40] Morisco, F.; Vitaglione, P.; Amoruso, D.; Russo, B.; Fogliano, V. and Caporaso, N. (2008). Food. Liver. Health. Molecular. Aspects. *Medicine.*, 29;144-150.

[41] Ahmed, M.M. and Zaki, N.I. (2009). Assessment the ameliorative effect ofpomegranate and rutin on chlorpyrifos-ethyl-induced oxidative stress in rats. Nature. Sci., 7:49–61.

[42] Vega, M.A. and Corbi, A.L.(2006). Human macrophage activation: too many functions and phenotypes for a single cell type., *Immunologia*.,25:248-272.

[43] Suzuki, T.; Yanai, M.;Yamaya, M.; Satoh-Nakagawa, T.; Sekizawa. K.; Ishida,S. and Sasaki, H.(2001). Erythromycin and common cold in COPD. *Chest.*, 120(3):730–733.

[44] Aseel MahmoodJ awad, Nagham Mahmood Aljamali, Saher Mahmood Jwad, Aseel M J, Saher M J, Development and Preparation of ciprofloxacin Drug Derivatives for Treatment of Microbial Contamination in Hospitals and Environment, *Indian Journal of Forensic Medicine & Toxicology,* 2020,14, 2, p:1115-1122.

[45] Tzulker, R.; Glazer, I.; Bar-Ilan, I.; Holland, D.; Aviram, M. and Amir, R. (2007). Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *J. Agric. Food. Chem*., 55: 9559–9570.

[46] Imad Kareem AlwanAlsabri, Hasaneen Kudhair Abdullabass, Nagham Mahmood Aljamali, Imad K A A, Hasaneen K A.,(2020), Invention of (Gluta.Sulfazane-Cefixime) Compounds as Inhibitors of Cancerous Tumors., *Journal of Cardiovascular Disease Research*, 11, 2.

[47] Price, C.D.; Yang, Z.; Karlnoski, R.; Kumar,D. and Chaparro, R.(2010). Effect of continuous infusion of asialoerythropoietin on short-term changes in infarct volume, penumbra apoptosis and behavior following middle cerebral artery occlusion in rat. *J. Clin. Exper, Pharmaco. Physiolo.*, 37: 185-204.

[48] Bosman, D.R. Winkler, A.S.; Marsden, J.T.; Macdougall, I.C. and Watkins, P.J. (2001). Anemia with erythropoietin deficiency occurs early in diabetic nephropathy. *Diabetic. Care.*, 24(3):495-499.

[49] Vidal, A.; Fallarero, A.; Pena, B. R.; Medina, M. E.;Gra, B.; Rivera, F.; Gutierrez, Y. and Vuorela, P. M. (2003) Studies on the toxicity of *Punicagranatum* L. (Punicaceae) whole fruit extracts. *J. Ethnopharmacol.* 89:295-300.

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