Evaluation the Effects of Three Types of Pomegranate Fruit Extracts on Some Biochemical Markers and Cytogenetic Parameters in Mice Administrated With Iron Dextran to Iron Overload

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Abstract

This study designed to evaluate the deleterious effects of iron overloaded on organs weights , enzyme activity of liver and bilirubin, iron concentration, total iron binding capacity in serum and on bone marrow cells proliferation in mice.

The obtained results showed non significant differences in organ weights (spleen and kidney) in group 1 that treated with three parts extracts of pomegranate except the decreasing in spleen weight subgroup 1-4 , significant increasing in liver and kidney weight in all animals treated with iron dextran (group 2) in comparison with their weights of organs in negative control (group 1). While significant increasing in iron concentrations in serum of animal administrated peel and juice in comparison with their positive control, furthermore , a significant increasing in its concentration extract in serum of animal that induced iron overload. Furthermore the increasing were significant in TIBC in serum of group 2 and that level were reduced to 83.1 % ,33% and 67.1% in serum of mice post treated with seed, peel and juice respectively , and the level of TIBC were at its lowest recorded in serum of group 1, while the highest TS% were recorded in group 1 ,in contrast group 2 that revealed lower TS% especially in animal administrated iron dextran only.

The study also showed significant increases in the level of AST and ALP activity and bilirubin in serum of almost all animals administrated by the three typesof pomegranate extracts,but the results also illustrated that the extracts could be reduced the highest levels of AST, ALP and bilirubin in serum of the animals with iron overload when administrated post treatment.

On the other hand, the results illustrated that the juice extract induced significantly bone marrow cells proliferation in group 1 and the animals administrated iron dextran, while the administrated animals with peel and seed extracts in group 2 reduced their bone marrow cells proliferation significantly in comparison with the positive control. Non significant differences were recorded in total chromosomal aberration in bone marrow cells in group 1, while the CAs revealed significant differences in group 2 especially in animals administrated iron dextran and the type of aberrations represented was mainly of chromatid break, dicentric, a centric chromosome and ring chromosome.

Keywords: Pomegranate, Peel, Juice, seed, Liver function tests, Mitotic index, Bone marrow cells proliferation.

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تقييم تأثير مستخلصات ثلاث أجزاء مختلفة لثمرة الرمان في مستوى بعض المؤشرت الكيموحيوية والوراثة الخلوية في فئران مجرعة بدكستران الحديد لاستحثاث فرط الحديد

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الخلاصة:

صممت هذه الدراسة لتقييم الضرر الناتج عن فرط الحديد في وزن الاعضاء ، فعالية انزيمات الكبد والبلروبين، تركيز الحديد ، سعة ارتباط الحديد الكلي في المصل وتضاعف خلايا نخاع عظم الفئران .

أظهرت النتائج فروقا غير معنوية في اوزان الاعضاء (الكبد ، الطحال، الكلى) في مجموعة الحيوانات الاولى المجرعة بثلاث اجزاء من مستخلصات ثمرة الرمان باستثناء الانخفاض في وزن الطحال في تحت المجموعة 4-1. وسجلت زيادة معنوية في اوزان الكبد والكلى في حيوانات المجموعة الثانية والمحث فيها فرط الحديد قياسا" بأوزان الاعضاء في حيوانات السيطرة السالبة (المجموعة الاولى).

اظهرت النتائج ارتفاع معنوي في تركيز الحديد في مصل الفئران المجرعة لمستخلص قشور وعصير الرمان قياسا" بمستواه في السيطرة السالبة مع زيادة واضحة في تركيز الحديد في مصل الفئران المحث فيها فرط الحديد وانخفاض هذا المستوى من تركيز الحديد لهذه الحيوانات (المجموعة الثانية) بتجريعها مستخلص البذور وعصير الرمان بينما ارتفع مستوى الحديد معنويا" بتجريع تلك الحيوانات لمستخلص القشور .فضلا" عن الزيادة المعنوية في مستوى TIBC في مصل حيوانات المجموعة الثانية والتي انخفضت بمقدار 83,1 % و33% و67% بتجريع الفئران بعد تلك المعاملة (استحثاث فرط الحديد) بمستخلص البذور ،القشور وعصير الرمان على التوالي. بينما سجلت اعلى نسب لتشبع الترانسفرين في مصل الفئران المجموعة الاولى قياسيا" الى المستويات المنخفضة من تلك النسب المسجلة في المجموعة الثانية.

كما سجلت نتائج الدراسة زيادة معنوية في مستوى فعالية AST و ALP والبلروبين في مصل اغلب الحيوانات المجرعة بالثلاث اجزاء من مستخلصات الرمان قياسا" بالسيطرة السالبة مع زيادة معنوية عالية لتلك الانزيمات في مصل الحيوانات ذات فرط الحديد ، وملاحظة قابلية تلك المستخلصات على خفض مستويات فعالية تلك الانزيمات والبلروبين عند تجريعها للحيوانات المحثة بفرط الحديد.

ومن جهة اخرى ظهر ان مستخلص عصير الرمان يحث خلايا نخاع العظم على التضاعف معنويا سواءا" كان بمفرده او عند تجريعه للحيوانات المحثة لفرط الحديد (بواسطة دكستران الحديد)، بينما سجل مستخلص البذور والقشور قابلية لتخفيض تضاعف خلايا نخاع العظم لمجموعة الحيوانات الثانية ولم تسجل فروقا معنوية في الزيغ الكروموسومي لخلايا نخاع العظم في المجموعة الاولى قياسا" بحيوانات السيطرة السالبة ، بينما ظهر زيغ كروموسومي معنوي في نخاع عظم المجموعة الثانية والاكثر وضوحا"في الحيوانات ذات فرط الحديد تمثل بكسور كروماتيدية ، كروموسومات ثنائية السنترومير وخالية السنترومير والكروموسوم الحلقي .

الكلمات المفتاحية : الرمان ، قشور، عصير ، وظيفة الكبد ، معامل انقسام الخلايا ، نخاع العظم

1. Introduction:

The pomegranate *(Punica granatum*L.) is one of the oldest edible fruits and is widely grown in many tropical and subtropical countries [1] .The first nation of the pomegranate is the Iranian plateau and Himalayas in north Pakistan and north India. Pomegranate is a juicely fruit , its juice provides about 16% of an adults vitamin C requirement per 100 ml and good source for vitamin B_5 , potassium and polyphenols ,such as tannins and flavonoids . The most abundant polyphenols in pomegranate juice are the hydrolyzable tannins called ellagitannins formed when ellagic binds

with carbohydrate [2]. Furthermore, Pomegranate seeds are rich in sugars, vitamins, polysaccharides, polyphenols and minerals.They have low oil content but are rich in polyunsaturated fatty acids [3].

Different types of Pomegranate has been used extensively in folk medicine for a number of therapeutic purposes[4] . Metabolites of pomegranate juice ellagitannins localize specifically in the prostate gland , colon and intestinal tissue .Clinical studies of pomegranate juice or fruit extracts revealed important usage of its against several diseases , as well as roots, bark and are used in the treatment of colic , colitis-diarrhia, dysentery, leucorrhia, menorrhagia, oxyariasis, paralysis , rectocele and headaches in traditional medicine [5]. Also, pomegranate fruit peel exerted diverse pharmacological functions as antioxidant activity [6], cytotoxic activity [7], hepatoprotective activity [8] and hypoglycemic activity [9].

Iron is the most abundant metal in the human body and it is an essential element for all life forms. Iron (Fe) has important functions in the body as a component of hemoglobin and numerous other iron containing proteins such as myoglobin and cytochrome-C, iron is stored mainly in the liver within the iron storage proteins, ferritin and haemoglobin [10].

Iron overload indicates accumulation of iron in the body due to any cause haemochromatosis. Haemochromatosis is mostly defined as iron overload with a hereditary/primary close [11] or originating from metabolic disorders. In general ,the term haemochromatosis is used to indicate the pathological effect of iron accumulation in any given organ [12]. Organs commonly affected by haemochromatosis are the liver ,heart ,and endocrine glands and on longer term lead to various diseases [13].

Iron overload is a serious chronic condition that develops when your body absorbs too much iron over many years .When the body stores excess iron, this is referred to as iron overload. Iron overload can have many causes, the most common being genetic. Other causes include too much iron in the diet (particularly from supplementation, chronic transfusion therapy, iron injections, chronic hepatitis, and other disorders [14].

Iron deficiency generally develops slowly and may not be clinically apparent until iron stores exhausted at the supply of iron to the tissue is compromised, resulting iron deficiency. The common cause of blood lose and iron deficiency in developed countries in intestinal parasitic infections, as well as the increased incidence of infectious disease associated with iron deficiency has been attributed to the impairment of the activities of iron containing enzymes in cells of immune system.

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical conditions including liver diseases [15]. Therefore , the aim of this study was to evaluate the effect of some parts of pomegranate fruit extractions on organs weight (kidney, liver, spleen) , serum iron,total iron binding capacity (TIBC) and transferring saturation TS% , some of liver enzymes in serum and proliferation of bone marrow cells in mice induced iron over load by iron dextran.

2. Materials and Methods

Almost all chemical was purchased from Fluka or BDH Company, colchicine purchased from local pharmacy produced by Ibn Hayan Company (Syria), and iron dextran (stock solution 100 mg/ml) from Dutchfarm, iron dextran diluted to appropriate concentration to obtain 12.5mgFe/100g of mice body weight [16].

3. Pomegranate extracts preparation:

A. Juice extract

The fruits were cut into pieces, the rind was removed and the seeds were separated . The juice filtered and sterilized by passing it through milipore filter unit (0.45 µm) then juice distributed in petri dishes and concentrated by oven at (37-40)^{o}C.

B. Peel and seed extracts

The fruit peel and seeds were dried in oven at (30-37) C^{o}, and then grounded. About 50 g of peel or seed powder mixed with 350 ml of 80% ethanol for 6 hours. The mixture centrifuged by cooling centrifuge at 3000 rpm for 15 minutes [17]. The supernatant were filtered and distributed in petri dishes and concentrated in oven at (37-40) C^{o}.

3. Experiment design and Biometry:

Forty males of albino Swiss mice (*Mus musculas*) their age ranged from 8 - 12 wk were obtained from biotechnology Research Center, Al-NahrainUniversity carried from Desember 2010 to March 2011 in animal house of Biotechnology research center, the animals were used in this study divided mainly into two groups ,each group 20 mice and were divided into four subgroup (each one 5 animals) as follows and placed in a separated cage in animal

house at room tempreture (25C^{o}),the animals were fed a suitable quantity of complete diet in addition to water , and treated as follow:

- **Group 1:** The first main group -20 animals- (were un administrated iron dextran) ,but divided into four subgroups represented by 1-1, 1-2, 1-3 and 1-4.

Subgroup 1-1 were injected intraperitonially (I.P) with 0.1 ml of normal saline (as negative control).Each animals of the subgroups 1-2,1-3 and1-4 were injected with 0.1 ml of 1/40 diluted seed,peel and juice extracts for 5 doses/week , respectively.

- **Group 2**: 20 mice were prepared from administrating iron over load by treated the animal with iron dextran at concentration of 12.5 mg Fe/100g of body weight for two weeks (5 doses /week) , each mouse administrated in each dose 3.13mg Fe / dose prepared from stock solution (100mg Fe/ml), then the animals were left for one week without any treatment .After that period the group were divided into four subgroup (each one 5 animals) represented as 2-1, 2-2, 2-3, and 2-4.

Subgroup 2-1 were injected intra- peritonially (I.P) with 0.1ml normal saline for additional 1 week by (5 doses/week as positive control).

Subgroup 2-2 , 2-3 and 2-4 were injected with 0.1 ml of 1/40 diluted seed, peel and juice extracts for 1 week (5 doses /week).

At the end of the experiment , The blood was obtained by puncture of heart, centrifuged at 120G for 15 mins and the animals were sacrificed .The liver, spleen and kidney were immediately excised and weighted , the serum was stored at - 80 ^{o}C, and then used to determine:- Serum Iron concentration, was calculated according to REF 92180 Kit (BIOLABO).

Total iron binding capacity (TIBC) was estimated with direct method by REF 92308 Kit (BIOLABO reagents Kit) and TS% was calculated by formula:

$$\text{Transferring Saturation TS\%} = \frac{\text{Serum Iron}}{\text{Total iron binding capacity}} \times 100$$

Furthermore , specific activities of liver enzymes which included : Aspartate transferase (AST) and Alkaline phosphatase (ALP) were determined with RNADOX and BIOLABO kits respectively, total bilirubin was determined with SPINREACT kit by using colorimetric methods.

Chromosomal preparation from somatic cells of mouse bone marrow:

Bone marrow cells were obtained from femur bone of mice scarified by cervical dislocation after 1.5 hour of injected (IP) with 0.25 ml colchicines. This method was done according to [18], the cells were fixed by fixative solution methanol: glacial acetic acid (3:1), finally cell suspension were dropped on chilled slide and dried at room temperature, then stained with Giemsa stain and washed with distilled water.

The cells were examined under light microscope (40X), and 1000 of divided and non-divided cells were counted for mitotic index (MI) and blast index (BI) and the percentage of cells at metaphase or blast stage was calculated according to the following equation:-

$$\text{Mitotic Index} = \frac{\text{Number of divided cells in metaphase}}{\text{Total number of divided and undivided cells (1000)}} \times 100$$

$$\text{Blast Index} = \frac{\text{Number of blast cells}}{\text{Total No. of cells(1000)}} \times 100$$

To estimate the chromosomal aberrations, the prepared slides were examined under oil immersion lens ,then for 25 divided cells at metaphase stage of mitotic division were examind for chromosomal aberrations.

Statistical analysis: The results were analyzed using the (completely randomized design – CRD) to evaluate the effect of extracts administration with and without iron overload on iron level in serum and proliferation of bone marrow cells of mice, the significant differences were compared between the treatments by (least significant differences test –LSD).(T-test) p-value < 0.05 was considered significant, All statistical analysis were done by using SPSS program,version-10 [19].

4. Results and Discussion:

Effect of three parts of pomegranate extracts in organ weights (Liver, Spleen and Kidney):

The results showed non significant differences in the organs weights (liver, spleen and kidney) in group 1 that treated with the three types of pomegranate extracts except a significant decreases in the spleen weight in subgroup 1-4 in compression with the negative control as clearly represented in table -1. Furthermore , the obtained results showed significant increases in the liver and kidney weights of animals treated with iron dextran

(positive control) in comparison with negative control , and non significant differences in organs weight in animals treated with iron dextran then administrated seed, peel and juice extracts (post treatment) in comparison with positive control.

The majority of functional iron within the body is present in haem proteins,such as haemoglobin ,myoglobin and cytochromes, although iron is an essential nutrition element for all lifes forms, iron overload may lead to various diseases caused by the accumulation of iron in the body [20]. Iron as we know stored mainly in the liver within the iron storage proteins ,ferritin and haemosidren ,many of key biological functions of iron in living system rely on the high redox potential. In contrast,other researchers have reported that parenteral iron-overload in rats increased the liver iron content mainly by deposition of iron in mitochondria that affected on oxygen transport or mitochondria electron as potentially harmful in terms capacity of oxidative damage to cellular component such as fatty acid ,proteins and nucleic acid (10).

Table 1- Influence of three types of pomegranate extracts on organs weights (Liver, spleen and kidney) in mice with and without iron overload.

	Groups	Liver wt. (g)	spleen wt. (g)	kidney wt. (g)
Group 1	Control (-ve C)	1.76±0.11c	0.23±0.11a	0.17±0.05 b
	Seed (1-2)	2.05±0.3c	0.3±0.08a	0.25±0.05 b
	Peel (1-3)	1.79±0.33c	0.29±0.06a	0.23±0.01 b
	Juice (1-4)	1.6±0.19c	0.19±0.02 b	0.18±0.05 b
Group 2	Fe (+ veC)	2.5±0.7a	0.28±0.07a	0.8±1.05a
	Seed (2-2)	2.31±0.22 a	0.22±0.03a	0.24±0.02 b
	Peel (2-3)	2.25±0.38 b	0.20±0.02a	0.23±0.01 b
	Juice (2-4)	2.47± 0.6a	0.25±0.06a	0.26±0.05 b

*** p<0.05, NS: non-significant / ****
Each value =Mean ±Std. Deviation

Organs commonly affected by iron overload disorders are liver, heart, endocrine glands ,pancreas ,bones and joints. [20]. Recently studies revealed pomegranate extracts (particularly peel) significantly reduced the damaging effects on the liver , and that extract lead to normal liver architecture suggesting, they may act as hepatoprotective food supplements [21].

Abnormal liver function is a well recognize complication of clinical states of iron overload such as many hematological disease , so our results revealed increasing in liver and kidney weight of almost all animals administrated in iron dextran either treated or un treated with pomegranate extracts caused by accumulation of iron in liver which finally lead to fibrosis and improvement in kidney structure [22], in additional to many researchers showed that iron accumulation damages the lysosomal membrane, releasing acid hydrolases into the cytoplasm and thus initiating cell damage.

Effect of different types of pomegranate extracts on serum iron concentration and Total iron binding capacity (TIBC) in mice

The results showed a significant increases in iron concentration in the serum of administrated animals with peel and juice only (287.8 and 503.3) µg /dl respectively in comparison with seed extract 254.5 µg /dl and negative control 112.2 µg /dl , and a significant increases in iron concentration in the serum of animals treated with iron dextran alone 525 µg /dl in comparison with iron concentration in the serum of negative control, furthermore a significant decreases in iron concentration in serum of mice post treated with seed extract (305.6 µg /dl) and juice extract (376.6 µg /dl) and high significant in increasing in the Fe concentration of serum of animals post treated with peel extract (1148.85 µg /dl) in comparison with positive control (figure -1)

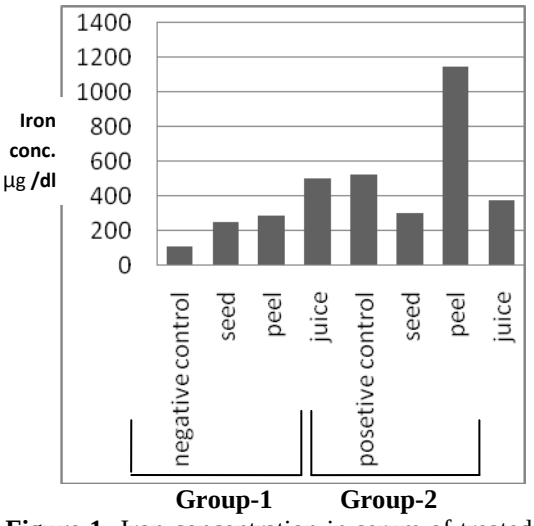

Figure 1- Iron concentration in serum of treated and untreated mice with iron dextran and three types of pomegranate extracts.

The results in figure -2 showed a significant increases in TIBC in serum of animal administrated peel and juice extracts (432 and 726) µg /dl respectively of pomegranate only in comparison with negative control 241.5 µg /dl ,while the obtained TIBC was highest 8914 µg /dl in animal treated with iron dextran only, and the levels of TIBS was decreased more significantly in subgroups of the animals post treated with the three extracts of seed, peel and juice (1505 , 5949 and 2391 µg /dl) respectively in comparison with positive control.

Another effect was shown in the TS% which recorded 46.58% in negative control and increased significantly in mice that administrated seed, peel and juice extracts (78.5% , 66.6% and 69.3%) respectively , while the TS% was reduced significantly to 5.8% in positive control , and it increased significantly only (20.3% , 19.3% and 12.8%) in serum of mice post treated with seed ,peel and juice extracts respectively.

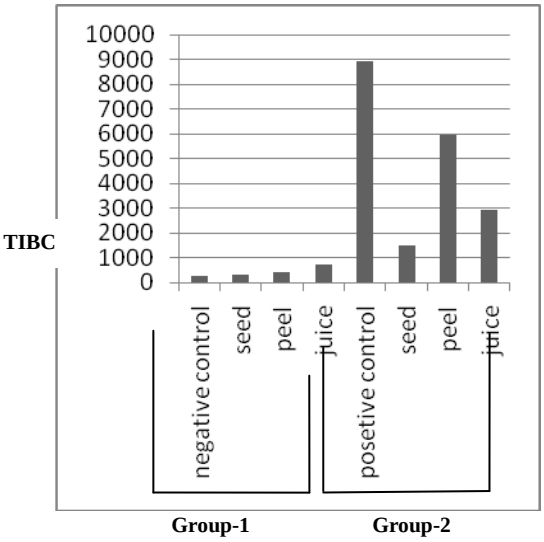

Figure 2- Total iron binding capacity (TIBC) in the treated and untreated mice with iron dextran and three types of pomegranate extracts.

Iron toxicity occurs when there was free iron in cell, which generally occurs when iron level exceeds the capacity of transfferin to bind to iron [23]. The content of iron binding galloyl groups may be the major determination of the inhibitory effect of phenolic compound, however, condensed of tannins do not interfere with iron absorption.

Peel and seed extracts contains tannins which inhibits the absorption of minerals such iron which may if prolonged lead to anemia, Tannins was metal ion chelators and tannins-chelated metal ions is not bioavailable by its interfere with iron absorption through complex formation with iron when it was in the gastrointestinal lumen leads finally to decrease bioavailability of iron [24].

There is an important differences in the way in which the phenolic compound interact with different hydroxylation patterns (gallic acid, catechin, and chlorogenic acid) and the effect on the iron absorption [25].

Generally increasing in iron concentration and TIBC may be refers to haemochromosis that was an autosomal disorders, in which in appropriate high absorption of dietary iron eventually lead to iron accumulation in tissue paranchymal cells and severe organ damage from iron at least during early stages of disease [26].

The effect of pomegranate administration in enzymes activity of liver and bilirubin concentration in serum.

The results showed significant increases in activity level of AST in serum of animals treated with (seed, peel and juice) extracts only in comparison with the negative control 5.58 IU/L (table-2) .On the other hand, the results recorded clearly significant decreases in the AST level in serum of groups 2 or post treated with (seed, peel and juice) extracts , in comparison to its level in serum of animal with iron load 136.522 IU/L (positive control).

Furthermore, the data recorded in table -2 revealed a significant increases in the activity level of ALP in serum of animal treated with seed and peel extracts (62.7 and 74.8)IU/L respectively in comparison to negative control (25.1 IU/L) , while the effects of inducing iron overload by administrating iron dextran were clearly significant in recording increased in activity level of enzyme in serum of animals in positive control (219.5 IU/L) in comparison to its level in negative control, and revealed significant decreasing in its activity level in serum of group 2 (post treatment) particularly in administrated peel extract (88.6 IU/L) and seed extract (160.2 IU/L) , in contrast the post treatment of juice (190.2 IU/L), juice extract showed a slight decreases in activity level of ALP in serum of that subgroup.

The results also revealed a significant increases in total bilirubin in serum of animals administrated with peel and juice extracts (1.04 and 0.78) mg/dl respectively , in comparison with its concentration in negative control (0.42 mg/dl). While the animals post treated with

(seed ,peel and juice extracts) showed significant decreases in the bilirubin concentration**(** 1.18 , 0.68 and 1.62) mg/dl in serum of group 2 that adminstrated to induce iron over load (2.16 mg/dl) .

AST and ALP serves as biomarker for liver function showed significant increase in all mice treated with extracts and iron dextran. The study showed that the highest evaluated levels of serum enzymes AST and ALP indicated to cellular leakage and loss of functional integrity of cell membrane of liver cells, furthermore, the concentration of bilirubin. Our results indicated that extracts preserved the structural integrity of hepatocellular membrane and liver architectural which was confirmed by histopathological studies (unpublished data).

Table 2- Enzymes activity and bilirubin concentration in serum of mice administrated three types of fruit extracts alone or post treated with iron dextran.

	enzymes / activity Animal groups	AST IU/L	ALP IU/L	Bilirubin mg/dl
Group 1	(-ve C)	5.58 ± 0.75 e	25.1 ± 2.1 d	0.42 ± 0.24 c
	Seed	28.12 ± 2.8 d	62.7 ± 3.8 c	0.66 ± 0.11 c
	Peel	41.7 ± 1.9 c	74.8 ± 2.9 c	1.04 ± 0.14 b
	Juice	38.82 ± 3.1c	29.8 ± 5.4 d	0.78 ± 0.21 c
Group 2	(+veC)	136.52 2 ± 5.8 a	219.5 ± 9.8 a	2.16 ± 0.41 a
	Seed	44.64 ± 2.4 c	160.2 ± 6.2 b	1.18 ± 0.46 b
	Peel	21.06 ± 1.8 d	88.6 ± 4.5 c	0.68 ± 0.21 c
	Juice	65.82 ± 4.2 b	190.2 ± 9.6 a	1.62 ± 0.17 b

Each value = mean ± SD, Small letters significant p< 0.05

These results may be due to hepatocellular necrosis which causes increase in the permeability of cell membrane resulting in the release of these enzymes in the blood stream. Furthermore , the affects of pomegranate extracts have a hepatopreventive capacity confirmed by reducing the liver damage caused by iron in especially in post treatment that recorded lower AST,ALP in comparison with positive control and may be increased the activity of GST of the liver ,brain and spleen [2] [27]. On the other hand , level of plasma bilirubin was increased carefully in mice administrated iron dextran and this refers to an increases in oxidative stress which may be due to a decrease in antioxidant defenses or due to an increase in the processes that produce oxidants [28] . A separate study in rats treated with CCl4- induced liver damage demonstrated pretreatment with a pomegranate peel extract (PPE) enhanced or maintained the free-radical scavenging activity of the hepatic enzymes catalase, super oxide dismutase, and peroxidase, and resulted in 54% reduction of lipid peroxidation values compared to controls. [29]

Influence of three types of pomegranate extracts and iron dextran on bone marrow cells proliferation

Mitotic and blast index

The mitotic index of bone marrow cells in negative control of mice was 25% as shown in table-3, however neither the administration of peel and seed extracts alone (22% and 18%) respectively, nor their administration post treatment caused significant differences in the MI (29% and 19%) respectively , compared with negative control ,In contrast the results revealed significant increasing in MI in bone marrow cells of animals administrated juice extract alone 47% or post treatment with juice extract after administrated iron dextran (59%) in comparison with negative control (25%) and positive control (41%) , the post treatment with peel and seed extracts reduced the MI significantly to 19% and 29% respectively compared to positive control.

These results indicated that seed and peel extracts caused inhibition of mitosis in bone marrow cells which was either due to altered arrangement of spindle microtubules or to their effects on the essential formation for completing metaphase [30], while the significant increases in the MI was induced after treatment with juice extract that may contain active constitute which may have stimulate or modulate effects on the mitosis process [31].

The blast index of mice bone marrow cells in negative control was (20%) as shown in table-3 ,The administration of peel and seed extracts did not cause significant differences in BI of mice bone marrow cells as compared with negative control, while the administration with juice extract caused significant increasing in BI (38%) compared to negative control.

Furthermore, the increasing in BI caused by administration with iron dextran (37%),this result was reduced significantly by post treatment with peel (21%) and seed (25%) extracts, and non significant decreases illustrated with the juice extract (28.6%). The decreasing in BI index may be due to the presence of active compound in these extracts which could stimulated blastogenesis or blast formation of bone marrow cells or their components delay the mechanisms and or preparation of main molecules that required for cell division. One of their molecules is Ribonucleotide reductase which was an iron depending enzyme that is required for DNA synthesis that iron was required for a number of vital functions including: growth, reproduction, healing and immune functions [32].

Table 3- Effect of three types of pomegranate extracts on mitotic and blast index in bone marrow cells of mice administrated extracts alone or post treated with iron dextran.

Animal group	MI %	BI %
Negative control	25 ± 2.5 c	20 ± 2.1 b
Seed	18 ±1.9 c	16 ± 2.6 c
Peel	22 ± 3.8 c	26 ± 1.9 b
Juice	47 ± 4.1 a	38 ± 3.2 a
Fe dextran 12.5 Fe/100g B.W.	41 ± 2.7 b	37 ± 4.5 a
Seed	29 ± 1.8 c	25 ± 3.4 b
Peel	19 ± 2.9 c	21 ± 3.7 b
Juice	59 ± 5.2 a	28.6±4.1 b

Chromosomal aberration:-

The study of total chromosomal aberrations (CAs) represented in table -4, non significant differences in mice groups administrated with seed ,peel and juice extracts (0.18 ,0.16 and 0.28) respectively . The main CAs represented as chromatide break, dicentric and centric chromosome in the negative control (0.27%) ,while the CAs in group 2 recorded significant increases in the aberration in bone marrow cells of mice treated with iron dextran (1.04%) illustrated in chromatide break, dicentric and acentric chromosome, chromosome break and ring chromosome. Most of these CAs were decreased significantly when animals post treated with seed, peel and juice extracts which reached to (0.33%, 0.56% and 0.54%) respectively.

Our results revealed that total chromosomal aberration in groups treated with pomegranate extracts only or post treated with iron dextran was lowered , our result could concluded that the presece of active constitutions particularly in seeds and peel extracrs that had biomarker characterization such as antioxidant activity ,scavenger of free radical roots by prevent their effects causes to inversion of chromosomes and results dicentric and acentric chromosome and reduce the lipid peroxidation ,and prevent the LDL level and cleavage of reactive peroxide chain as Tuck *et al* mentioned [33].

Excessive iron can be toxic because free ferrous ion reacts with peroxides to produce free radicals, which was highly reactive and can damaged DNA, protein, lipids and other cellular components [27].The main ability of polyphenols and tannins (metal ion chelators) that contains in seed and peel extracts play important role in scavenger free radical molecules by chelating iron ions in its parts of catechol moietics which forms a complex with Fe^{+} at physiological pH and reduce the reverse reaction of hydrogen peroxide to hydroxyl group as illustrated by [34].

We concluded to the patients whom have iron overload (according to our experiments in mice):

1- To prevent or take away from the pomegranate juice in their diet, but our result advice to supplementing or inrichment their diet with pomegranate peel .

2- Because AST and ALP serves as biomarkers for liver function, it could be used as biochemical markers for liver cells damage, our results advice to use pomegranate peel extract to improve or preserve the functional and structural integrity of cell membrane of liver cells.

3- Our results indicates that pomegranate juice induced the mitotic and blast index in bone marrow cell, thus it might be play as inducer factor for their proliferation , in contrast the administration of the seed extract to animals that induced iron overload by iron dextran showed reducing in their cytogenetics parameters represented by mitotic and blast index , as well as CAs.

Table 4- Chromosomal aberration (CAs) in bone marrow cells of mice administrated three types of pomegranate extracts alone, or post treated with pomegranate extracts.

Animal Groups / Treatment		Chromosomal aberrations %					
		Chromatids break	**Dicentric**	**A centric**	**Ring chromosome**	**Chromosome break**	**Total chromosomal aberrations**
Group 1	**Negative control**	0.06 ±0.005	0.07±0.01	0.07±0.004	0.04±0.001	0.03±0.01	0.27c
	Seed	0.06 ±0.006	0.04±0.007	0.04±0.007	0.02±0.001	0.01±0.003	0.18c
	Peel	0.02 ±0.007	0.05±0.002	0.06±0.008	0.008±0.003	0.02±0.004	0.16c
	Juice	0.07 ±0.008	0.06±0.004	0.08±0.006	0.02±0.005	0.05±0.003	0.28c
Group 2	**Positive control**	0.22 ±0.02	0.25±0.01	0.29±0.03	0.12±0.001	0.16±0.06	1.04a
	Seed	0.03 ±0.005	0.12±0.04	0.09±0.03	0.04±0.001	0.05±0.01	0.33c
	Peel	0.07 ±0.001	0.1±0.006	0.20±0.04	0.08±0.02	0.11±0.04	0.56b
	Juice	0.1 ±0.005	0.18±0.008	0.11±0.02	0.06±0.02	0.09±0.02	0.54b

Each value = mean ± SD, Small letters significant p< 0.05

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