The effect of chronic iron drug compound exposure on some biochemical values in ewes suffer from iron deficiency

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Abstract

The study was conducted to evaluate the effects of chronic iron toxicity on some biochemical parameters. Twenty Iraqi local breed ewes at age (2-4) year, clinically pregnant have different class of iron deficiency at the period from 25/10/2012 to 31/1/2013, animal divided into two groups, treated group treated with 10 mg/ kg of body weight iron dextran every week for 12 weeks, control group leave on its feed only. All animals monitored clinically during the study: Serum biochemical tests: total protein, albumin, globulin, cholesterol, alanine aminotransferase, serum iron, total iron binding capacity, Unbound iron binding capacity, transferrin saturation at the weeks (zero, 2, 4, 6, 8, 10, 12, 14) during the period of iron dextran injections. Serum biochemical tests result showed: that total protein of treated group showed significant increases ($p< 0.05$) reached 6.8g/dl at the weeks 2, and then a gradual decrease reached 4.3 at the weeks 14. While, ewes of control group increased reach 6.31 g/dl at weeks 2 and remained unchanged to end of study at the weeks 14. Albumin and globulin of treated group showed significant increase ($p<0.05$) at the weeks 2, and then gradual decrease to reached 2.4g/dl, 1.9g/dl respectively. While, ewes of control group showed a variable result which ranged (1.6-3.3) g/dl for albumin and (1-4.1) g/dl. Alanine aminotransferase of treated group showed a significant decrease reached 34.8 U/L at the weeks 12, and then a significant increase reached 43 U/L at the weeks 14. While, ewes of control group showed no significant difference and ranged (36-42 U/L). Cholesterol of treated group showed a gradual increase reached 110 mg/dl at the weeks 6 then gradual decrease to reached 19mg/dl at the weeks 14. While, control group showed significant increase at the weeks 6 then return to it is level (44-56) mg/dl. Glucose of treated group showed a significant increase reached 75.8 mg/dl at the weeks 10. While, the ewes of control group showed significant increase at the weeks 14 only. Serum iron of treated group showed gradual increase reach the peak 54 µmol / L at the weeks 4 then gradual decrease to reached 22 µmol/ L at the weeks 14. While, ewes of the control group showed gradual increase at weeks 2 reach 30 µmol / L and remained unchanged after that. Total iron binding capacity of treated group showed a gradual increase reached the peak 246 µmol/ L at the weeks 8. On other hand control group showed variable result range (47-97) µmol/L, depend on the normal range (42.9-74.1). Unbound iron binding capacity of treated group showed a gradual increase reached 140 µmol/ L at the weeks 10. On other hand ewes of control group showed variable result range (22-60) µmol/L. depending on the normal range (16.9-46.6) Transferrin saturation of treated group showed a gradual increase reach 45.2% at the weeks 6 then gradual decrease reached 20% at the weeks 10. On other hand control group showed variable result rang (32-59)%. According to these result it is concluded that: 10 mg/kg B.W iron dextran weekly for one month considers a good treatment for anemia but if continues treating could cause accumulation in the body and interfere with the body metabolisms.

تأثير التعرض المزمن بمركبات الحديد الدوائية على بعض القيم الكيموحيوية في النعاج التي تعاني نقص الحديد

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الخلاصة

الدراسة تجريبية هدفت الى معرفة تأثير التعرض المزمن لمركبات الحديد الدوائية على: بعض القيم البايوكيميائية. اجريت الدراسة بين الفترة 2012/10/25 الى 2013/1/31 على عشرون من النعاج المحلية العراقية وبعمر يتراوح من (2–4) سنة وبمراحل مختلفة من نقص الحديد. شطرت الى مجموعتين كل منها عشر نعاج، المجموعة الاولى المعاملة والتي عوملت باحدى مركبات الحديد والثانية تركت على العليقة فقط فالمعاملة تم حقنها باحدى مركبات الحديد بالعضل 10 ملغم/ كغم من وزن الجسم كل اسبوع ولمدة ثلاثة اشهر اما الثانية كما سلف تركت على العليقة. فحصت الحيوانات لكلا المجموعتية خلال الفترة اعلاه فحوصات المصل البايوكيميائية التالية: البروتين الكلي، الالبومين، الكلوبيولين، الكولستيرول، انزيم الأنين امينوترانسفريز، الكلوكوز، الحديد، القدرة الكلية للحديد المرتبط، القدرة للحديد غير المرتبط. اظهرت نتائج الفحوصات البايوكيميائية للمصل ما يلي: تركيز البروتين الكلي للمجموعة المعاملة اظهر ارتفاعاً معنوياً وصل إلى 6.8 غ/100 مل في الأسبوع الثاني بعد ذلك شهد انخفاضاً تدريجياً ليصل 4.3 في الأسبوع الرابع عشر. بينما الحيوانات غير المعاملة ازدادت لتصل 6.31 غ/100 مل ثم استقرت على هذا المستوى. وكان تركيز الالبومين والغلوبيولن ايضاً يشهد ارتفاعاً معنوياً في الاسبوع الثاني وبعد ذلك انخفض تدريجياً ليصل 2.4 غ/100 مل البومين، 1.9 غ/100 مل غلوبيولين وبعد الاسبوع الثاني عشر. بينما الحيوانات غير المعاملة اظهرت نتائج متذبذبة تراوحت بالنسبة للالبومين (1.6–3.3) غ/100 مل و1–4.1 غ/100 مل بالنسبة للغلوبيولين. انزيم الأنين امينوترانسفريز للمجموعة المعاملة فقد اظهر انخفاض معنوي في الاسبوع الثاني عشر وصل الى 34.8 وحدة/لتر ثم ارتفع ارتفاعاً معنوي بعد الاسبوع الرابع عشر وصل الى 43 وحدة/لتر. بينما الحيوانات غير المعاملة لم تظهر اختلافات معنوية. اما بالنسبة لتركيز الكولسترول للمجموعة المعاملة فقد اظهر ارتفاع معنوي وصل الى 110 ملغم/100 مل في الاسبوع السادس بعد ذلك اظهر انخفاض تدريجي ليصل الى 19 ملغم/100 مل بعد الاسبوع الثاني عشر. بينما الحيوانات غير المعاملة فقد اظهرت ارتفاع معنوي في الاسبوع السادس ومن ثم عاد الى مستواه بمدى 44–56 ملغم/100 مل. اما بالنسبة لتركيز الكلوكوز للمجموعة المعاملة فقد اظهرت ارتفاع تدريجي ليصل إلى 75.8 ملغم/100 مل في الأسبوع العاشر بينما الحيوانات غير المعاملة فقد اظهرت ارتفاعاً معنوياً فقط في الاسبوع الرابع عشر. وبينت نتائج الحديد في المصل للمجموعة المعاملة ارتفاع تدريجي ليصل اعلى مستوى له 54 مايكرومول/لتر في الاسبوع الرابع بعد ذلك اظهر انخفاض تدريجي ليصل الى 22 مايكرومول بعد الاسبوع الرابع عشر. بينما الحيوانات غير المعاملة فقد اظهرت ارتفاع تدريجي لتصل الى 30 مايكرومول/لتر ثم استقر على هذا المستوى. اما القدرة الكلية للحديد المرتبط للمجموعة المعاملة فقد شهدت ارتفاعاً تدريجاً ليصل اعلى مستوى له 246 مايكرومول/لتر في الاسبوع الثامن. انما الحيوانات غير المعاملة اظهرت نتائج متذبذبة بمدى 47–97 مايكرومول/لتر. اما قدرة الحديد غير المرتبط للمجموعة المعاملة فقد اظهرت ارتفاع تدريجي لتصل القمة 140 مايكرومول/لتر في الاسبوع العاشر. انما الحيوانات غير المعاملة اظهرت نتائج متذبذبة بمدى 22–60 مايكرومول/لتر. فكانت نسبة تشبع ناقل الحديد

للمجموعة المعاملة والتي اظهرت ارتفاع تدريجي لتصل إلى 45.2% في الأسبوع السادس بعد ذلك اظهرت انخفاض تدريجي لتصل الى 22% في الاسبوع العاشر. بينما الحيوانات غير المعاملة كانت النتائج متذبذبة بمدى 32−59%. نستنتج من الدراسة ان حقن الحيوانات 10 ملغم/ كغم لوزن الجسم من الحديد اسبوعياً لمدة شهر يعتبر علاج جيد لحالات فقر الدم لكن الاستمرار باستخدامه لمدة اطول يؤدي الى تراكمه في الجسم وبالتالي يؤثر سلبياً على فعاليات الجسم.

Introduction

Iron is an essential component not only for hemoglobin synthesis and erythropoiesis but also for many enzymes and hormones, iron deficiency had negative effect on the sheep flock, deficient iron causes anemia in all animals (1). Iron deficiency is common finding in ruminant, where low dietary intake, starvation, gastrointestinal parasites, blood parasite infection increased incidence of infectious disease, inadequate gastrointestinal absorption, hemorrhage and effect of pregnancy and lactation, all these causes influence the level of essential blood constituents especially iron, cobalt, copper and many biochemical functions such as iron utilization and hemoglobin synthesis, (2). Parenteral iron therapy is indicated in situations such as intolerance, contraindications or inadequate response to oral iron, however, parenteral iron is now a useful treatment in cases where there is a short time to surgery, severe anemia, especially if accompanied by significant ongoing bleeding, use of erythropoiesis-stimulating agents, etc. (3). Iron's toxicity is largely based on its ability to catalyze the generation of radicals, which attack and damage cellular macromolecules and promote cell death and tissue injury, where excessive iron accumulation results in tissue damage and organ failure, pathological iron accumulation in the liver has also been linked to the development of hepatocellular cancer (4).

Materials and Methods

- **Animals:** 20 Iraqi local breed anemic ewes at age (2-4) year were identified by ear tags. All animal monitored with Serum biochemical tests: total protein (TP), albumin, globulin, cholesterol, alanine aminotransferase, serum iron, total iron binding capacity, unbound iron binding capacity, transferrin saturation with (BIOLABO kit) according to 65 at (zero, 2, 4, 6, 8, 10, 12, 14) weeks.
- **Grouping of the animal:** The ewes where divided into two groups as the following: **Group 1** which considered as control group: consist of 10 ewes leave on its feed only, and subjected to all tests mentioned above. **Group 2** which considered as treatment group: consist of 10 ewes injected with iron dextran 10 mg/k of body weight intramuscularly every week for 3 month, and subjected to all tests mentioned above.
- **Statistical analysis**: All data are represented as means + SE. One way analysis of variance (One-way ANOVA) by using SPSS program, followed by Least Significant Difference (LSD) test were used to determine differences among means of investigated groups. The level of statistical significant was set at ($P<0.05$) (5).

Results

- **Biochemical analysis:**

1. Liver function test:

- **Total serum protein (Table 1):** Ewes of treated group showed a significant increase reached 6.8 g/dl at the weeks 2, and then showed a gradual decrease reached 4.3 g/dl at the weeks 14 of iron injection. While ewes of control group increased reach 6.31 g/dl at weeks 2 and remained unchanged to end of study at the weeks 14.

Table (1) effect of weekly intramuscular 10 mg/kg B.W iron dextran on serum Total protein (g/dl); mean ± S.E

Group / Time week	Control	Treated	Control		Treated	
			Min	Max	Min	Max
Zero	2.921± 0.310 Acb	3.820±0.511 Ac	2.28	3.94	1.70	7.36
2	5.757±0.235 Bab	6.837±0.313 Aa	5.07	6.52	5.84	9.00
4	5.829±0.534 Aab	5.734±0.285 Ab	4.06	6.98	3.62	6.87
6	6.313±0.242 Aa	6.102±0.177 Aab	5.81	6.95	5.38	7.05
8	6.320±0.088 Aa	5.978± 0.307 Aab	6.02	6.52	4.40	7.60
10	5.839± 0.270 Aab	5.428± 0.201 Ab	5.07	6.48	4.44	6.58
12	5.072± 0.177 Ab	5.207± 0.194 Ac	4.61	5.59	4.48	6.24
14	4.936± 0.316 Ac	4.344± 0.354 Ac	4.16	5.84	2.89	6.66

Capital letters denote the differences between groups, p<0.05.
Small letters denote the differences within group, p<0.05.

- **Serum Albumin (Table 2):** Ewes of treated group showed a significant increase at the weeks 2 reached 2.413 g\dl then remained unchanged to the end of study. On the other hand control group showed a variable result which ranged (1.6-3.3) g/dl.

Table (2) effect of weekly intramuscular 10 mg/kg B.W iron dextran on serum albumin g\dl; mean ± S.E

Group / Time week	Control	Treated	Treated		Control	
			Min	Max	Min	Max
Zero	1.823±0.301 Abc	1.477±0.586 Ab	0.57	2.43	1.20	2.97
2	1.611±0.248 Abc	2.413±0.308 Aa	0.90	4.21	0.85	2.28
4	3.312±0.276 Aa	2.928±0.253 Aa	1.91	3.93	2.43	3.93
6	3.092±0.173 Aa	2.925±0.249 Aa	1.93	4.52	2.62	3.59
8	2.908±0.134 Aa	2.918±0.141 Aa	2.29	3.74	2.62	3.34
10	2.875±0.323 Aa	2.742±0.073 Aa	2.28	3.10	2.09	3.79
12	2.064±0.235 Ab	2.539±0.107 Aa	2.10	2.92	1.24	2.69
14	2.663±0.300 Aab	2.436±0.092 Aa	2.09	2.76	2.00	3.76

Capital letters denote the differences between groups, p<0.05.
Small letters denote the differences within group, p<0.05.

- **Serum Globulin (Table 3):** Ewes of treated group showed a gradual increase at the weeks 2 reached 4.424 g/dl then a gradual decrease at the weeks 14 reached 1.9 g/dl. On the other hand control group showed a variable result which ranged (1-4.1) g/dl. And there was a significant difference between treated and control group.

Table (3) effect of weekly intramuscular 10 mg/kg B.W iron dextran on serum globulin (g/dl); mean ± S.E

Group / Time week	Control	Treated	Control		Treated	
			Min	Max	Min	Max
Zero	1.098± 0.220 Bc	2.343±0.390 Abc	0.57	1.81	1.12	5.54
2	4.146 ±0.396 Aa	4.424 ±0.370 Aa	3.11	5.06	3.25	7.04
4	2.116 ±0.623 Abc	2.805±0.272 Ab	0.02	3.78	1.36	4.00
6	3.221±0.191 Aab	3.176±0.285 Ab	2.75	3.89	1.56	4.47
8	3.412±0.125 Aab	3.059± 0.266 Ab	3.13	3.88	2.04	4.44
10	2.963± 0.383 Ab	2.685± 0.171 Abc	1.97	4.27	1.81	3.86
12	3.007± 0.181 Ab	2.668± 0.185 Abc	2.44	3.57	2.21	4.12
14	2.273± 0.394 Abc	1.908± 0.362 Ac	1.54	3.44	0.60	4.56

Capital letters denote the differences between groups, p<0.05.
Small letters denote the differences within group, p<0.05.

- **Serum Alanine aminotransferase activity (Table 4):** Ewes of treated group showed no significant difference at first, but showed a significant decrease at the weeks 12 reached 34.8 U/L and increase at the weeks 14 reached 43 U/L. on the other hand control group showed no significant difference ranged (36-42 U/L).

Table (4) effect of weekly intramuscular 10 mg/kg B.W iron dextran on activity of serum Alanine aminotransferase (U/L); mean ± S.E

Group / Time week	Control	Treated	Treated		Control	
			Min	Max	Min	Max
Zero	40.8± 2.152 Aa	41.38±2.04 Aa	27.25	48.5	33.25	46.25
2	38.3 ±3.175 Aa	41.2 ±0.578 Aa	38.25	43	26.25	45
4	37.45 ±1.977 Aa	37.58±1.534 Aab	32.25	48	31.5	42.25
6	40.14±2.545 Aa	39.14±1.504 Aab	35	50.75	34.25	49.5
8	40.35±0.923 Aa	39.3± 1.480 Aab	26.75	43.25	38.25	43
10	42.19± 0.916 Aa	38.7± 0.925 Bab	34.75	43	39.25	44.7
12	36.2± 2.971 Aa	34.87± 1.078 Ab	29	41.37	28.25	42.25
14	37.6± 0.744 Ba	43.05± 3.396 Aa	37.75	73.25	35.5	40

Capital letters denote the differences between groups, p<0.05.
Small letters denote the differences within group, p<0.05.

- **Serum Cholesterol (Table 5):** Ewes of the treated group Showed significant increase of cholesterol at the weeks 6 reached 110 mg/dl, then gradual decrease to the end of the study. On other hand ewes of the control group showed significant increase at the weeks 6 reached 123.95 mg/dl then return to it is level (44-56) mg/dl.

Table (5) effect of weekly intramuscular 10 mg/kg B.W iron dextran on serum cholesterol (mg/dl); mean ± S.E

Group / Time week	Control	Treated	Control		Treated	
			Min	Max	Min	Max
Zero	47.903± 9.948 Ab	43.036±6.753 Ac	28.92	76.63	13.49	80.00
2	97.156±41.831 Aab	87.566±8.894 Ab	39.52	263.61	46.75	127.71
4	91.373±3.987 Aab	103.084±8.334 Aab	83.86	105.06	55.42	137.83
6	123.95±15.494 Aa	110.84±8.849 Aa	83.86	176.87	72.29	149.88
8	71.304±8.795 Ab	60.217± 6.555 Ac	44.57	90.22	22.83	89.67
10	56.630± 1.936 Ab	57.934± 6.478 Ac	25.00	96.20	36.96	109.78
12	55.821± 8.797 Ab	69.076± 5.844 Ab	30.98	84.00	35.33	93.48
14	44.787± 0.236 Ab	19.343± 5.174 Bd	23.23	80.00	5.56	51.52

Capital letters denote the differences between groups, p<0.05.
Small letters denote the differences within group, p<0.05.

- **Serum Glucose (Table 6):** Ewes of treated group showed show slight decrease at the weeks 8 reached 55.465mg/dl and increase at the period (10-14) week and ranged (67.659-81.808) mg/dl. in other hand ewes of control groups showed significant increase only at the weeks 14 reached 78.510 mg/dl.

Table (6) effect of weekly intramuscular 10 mg/kg B.W iron dextran on serum glucose (mg/dl); mean ± S.E

Group / Time week	Control	Treated	Control		Treated	
			Min	Max	Min	Max
Zero	54.709± 1.645 Abc	56.453±2.343 Ab	50.58	59.30	50.00	70.35
2	58.488±4.408 Ab	54.854±1.283 Ab	50.00	69.48	50.00	63.37
4	67.616±2.928 Aab	67.994±2.013 Aab	61.63	77.33	59.59	81.40
6	61.681±1.177 Ab	60.613±0.633 Ab	57.50	64.77	57.27	63.18
8	63.540±3.795 Ab	55.465±1.421 Bb	55.59	75.47	50.00	62.42
10	62.795±3.461 Bb	75.838±11.808 Aa	54.35	74.84	55.59	180.43
12	59.219±3.000 Bb	67.659±4.112 Aab	50.00	66.31	54.96	89.72
14	78.510±9.088 Aa	81.808±5.138 Aa	50.00	103.90	60.28	110.28

Capital letters denote the differences between groups, p<0.05.
Small letters denote the differences within group, p<0.05.

2. Iron profile test:

- **Serum iron concentration (Table 7):** Ewes of treated group showed significant increase of iron concentration in serum with iron injection reach 54 µmol/ L at the weeks 4, and then showed gradual decrease reach 22 µmol/ L at the weeks 14. On other hand ewes of the control group showed gradual increase at weeks 2 reach 30 µmol/ L and remained unchanged after that range (18-30 µmol/ L), depending on the normal range (17.1-31.6) µmol/ L. And there was significant difference between treated and control group.

Table (7) effect of weekly intramuscular 10 mg/kg B.W iron dextran on serum iron (µmol/L); mean ± S.E

Group / Time week	Control	Treated	Control		Treated	
			Min	Max	Min	Max
Zero	18.510± 2.319 Ac	20.081±4.436 Ac	9.32	21.98	6.66	53.12
2	30.858±1.675 Aa	36.031±4.970 Ab	26.98	37.06	14.12	72.86
4	28.164±2.661 Bab	54.279±4.824 Aa	20.77	34.15	27.11	81.53
6	27.848±1.260 Bab	51.835±3.873 Aab	25.11	31.47	33.44	72.39
8	28.482±0.775 Bab	41.782± 4.607 Aba	26.45	30.91	19.38	58.15
10	26.985± 1.419 Aab	33.581± 3.745 Ab	23.03	30.03	10.72	47.20
12	29.121± 2.593 Aab	26.900± 2.610 Ac	24.11	35.47	15.58	42.29
14	23.542± 1.440 Ab	22.034± 2.623 Ac	20.11	27.35	6.84	35.00

Capital letters denote the differences between groups, p<0.05.
Small letters denote the differences within group, p<0.05.

- **Serum Total iron binding capacity (Table 8):** Ewes of treated group showed gradual increase to reach 246 µmol/L at the weeks 8. On other hand control group showed variable result range (47-97) µmol/L, depend on the normal range (42.9-74.1). And there was significant difference between treated and control group.

Table (8) effect of weekly intramuscular 10 mg/kg B.W iron dextran on serum total iron binding capacity (µmol/L); mean ± S.E

Group / Time week	Control	Treated	Control		Treated	
			Min	Max	Min	Max
Zero	47.250±12.166 Ac	51.530±3.221 Ac	20.81	76.28	39.01	70.38
2	97.141±4.748 Aa	130.38±22.055 Ab	78.98	104.92	61.55	290.17
4	50.870±5.682 Bb	118.376±11.551 Abc	38.00	70.17	44.47	170.33
6	72.171±17.029 Bab	154.63±21.400 Ab	36.06	121.42	96.42	330.74
8	81.250±12.306 Bab	122.19± 10.427 Abc	48.42	124.81	93.08	184.97
10	84.541± 6.695 Bab	246.16± 44.746 Aa	66.40	106.85	51.55	567.07
12	54.870± 5.682 Bb	128.29± 20.464 Ab	42.00	74.17	70.43	294.62
14	76.171± 7.029 Aab	62.619± 4.586 Ac	40.06	125.42	40.05	84.65

Capital letters denote the differences between groups, p<0.05.
Small letters denote the differences within group, p<0.05.

- **Serum unbound iron binding capacity (Table 9):** Ewes of treated group showed gradual increase to reach 140µmol/L at the weeks 10. On other hand ewes of control group showed variable result range (22-60) µmol/L. depending on the normal range (16.9-46.6). And there was significant difference between treated and control group.

Table (9) effect of weekly intramuscular 10 mg/kg B.W iron dextran on serum unbound iron binding capacity (µmol/L); mean ± S.E

Group / Time week	Control	Treated	Control		Treated	
			Min	Max	Min	Max
Zero	28.739± 0.999 Ab	32.615±3.378 Ac	0.32	54.30	13.55	44.61
2	66.283±6.383 Aa	97.062±22.847 Aab	41.92	77.94	15.54	257.14
4	22.706±7.481 Bbc	64.655±10.895 Ab	3.85	46.23	17.37	117.14
6	44.323±17.739 Aab	103.10±21.691 Aab	5.85	95.58	41.51	280.98
8	52.768±12.022 Aab	82.052±11.697 Ab	21.17	95.65	40.89	141.69
10	57.556±7.565 Bab	140.58±9.238 Aa	38.81	83.82	92.17	197.78
12	25.749±5.698 Bb	102.75±21.701 Aab	6.53	38.75	45.74	277.74
14	52.628±18.218 Aab	67.941±18.194 Ab	12.71	104.90	32.41	223.19

Capital letters denote the differences between groups, p<0.05.
Small letters denote the differences within group, p<0.05.

- **Serum Transferrin saturation (Table 10):** Ewes of treated group showed a significant increase reached 48.2% at the weeks 6 then gradual decreases reach 20% at the weeks 10. On other hand control group showed variable result rang (32-59)%, Depending on the normal range (30.3-60.7). And there was significant difference between treated and control group.

Table (10) effect of weekly intramuscular 10 mg/kg B.W iron dextran on serum Transferrin saturation (%); mean ± S.E

Group / Time week	Control	Treated	Control		Treated	
			Min	Max	Min	Max
Zero	49.174± 12.957 Aab	34.752±7.091 Aab	27.03	98.45	13.27	79.68
2	32.491±3.733 Ab	31.661±6.505 Ab	25.71	46.92	7.65	74.75
4	59.338±10.235 Aa	48.221±4.879 Aa	34.13	89.87	31.22	74.93
6	50.103±12.567 Aab	36.967±4.271 Aaba	21.28	83.77	15.05	63.43
8	38.066±5.305 Aab	34.957±4.976 Aab	23.37	56.27	12.50	58.71
10	32.964±3.536 Ab	20.773±8.108 Bbb	21.56	41.55	4.38	91.56
12	55.142±7.658 Aab	23.919±4.060 Bb	42.20	84.45	5.73	48.04
14	39.903±10.407 Aab	33.848±3.650 Aab	16.36	68.28	17.08	53.40

Capital letters denote the differences between groups, p<0.05.
Small letters denote the differences within group, p<0.05.

Discussion

- **Total serum protein:** These results of agree with (2, 6, 7, 8). The alterations in Total serum protein is dependent upon an understanding of the various physiological factors and pathological or induced factors that might cause such alterations, these alterations often due to a decrease in quantity of albumin (6). At first the increasing of total serum protein is due to increase metabolic activity and feed bioviability due to iron injection, Then Total serum protein decreased in animals due to liver effecting by accumulation of iron, since the majority of the decreases in Total serum protein is a direct reflection of hypo albuminemia (9).
- **Serum Albumin:** Albumin represents the major and predominant antioxidant in the plasma (10, 11). It represented 70% of the free radical tapping activity of serum (12). Iron injection resulted in significant decrease in the level of endogenous antioxidant like reduced albumin (13). Excess free iron is suggested to increase production of superoxide and hydroxyl radical via the fenton reaction and oxidative stress development (14). The observed decrease in serum albumin concentration after iron administration indicated oxidation of serum albumin in suggested that albumin has protective mechanism in response to iron induce ROS generation (15). Beside such observation could be ascribed to changes in protein and free amino acid metabolism and their synthesis in the injured liver cell and/or or increase protein degeneration (13). This decrease in catalase activity is due to iron toxicity lead to lose of NADPH lead to adversely affect erythrocyte enzyme activity (16). Albumin decrease in serum in iron toxicity, this result agrees with (17, 18, 19) on rats, rabbits and mice respectively .due to depletion of antioxidant status of the body (albumin). Sulfhydryl group antioxidant in plasma and extravascular space (20). It has been shown ROS production after iron exposure may mediate oxidation, proteolysis and poor degradation of albumin leading to it is depletion (21, 22).
- **Serum Globulin:** Alpha-1 Globulins the major protein associated with alpha-1 globulins is alpha-1 antitrypsin. Elevated levels may indicate an acute inflammatory disorder, while decreased levels may be indicative of Alpha-1 antitrypsin deficiency. Alpha-1 antitrypsin deficiency is a hereditary disease that is associated with chronic lung and liver disorders. According to the Alpha-1 Association, those who suffer from this disease have the potential of developing emphysema of the lungs, cirrhosis of the liver and liver cancer. And Alpha-2 globulins are primarily composed of two proteins, macroglobulin and haptoglobulin. Increased levels of macroglobulin are used as a diagnostic factor in nephrotic syndrome, which describes a condition of the kidneys where proteins are leaked from the blood into the urine. Macroglobulin has also been indicated in Alzheimer's disease. Detection of low levels of haptoglobulin, among other tests, is used in the diagnosis of hemolytic anemia, a condition that causes the destruction of red blood cells. And there are several types of beta globulins, but they all are primarily composed of transferrin. Transferrin tightly binds iron in the blood and transports it to cells with a transferrin receptor, where the iron molecule is then imported into the cell. Elevated levels of transferrin are often indicative of someone suffering from iron-deficient anemia. Conversely, decreased transferrin levels are associated with iron overload, or hemochromatosis. Transferrin is also used as a diagnostic for liver disease and protein malnutrition (23). But the levels and types of each one determined only by test called serum electrophoresis, so we cannot know the real cause of change in its concentration.
- **Serum Alanine aminotransferase activity:** These results of (ALT) are comported with (2); they reported that the increase in this enzyme activity suggests a liver an effect, and increase in the serum activity of these enzymes indicates hepatocellular

damage. On the other hand, increases in ALT activity have been reported on occasion with muscle diseases such as toxic myopathies, so ALT used as a muscle-specific enzyme in large animals because hepatic ALT activity in large animls is very low (24). Increased activity has been reported in myopthies of lambs, pigs and horses (25). (26) and (27) reported that increased ALT activity occurs with sublethal hepatocellular injury or necrosis which may occur due to iron accumulation, also muscle necrosis may cause increased serum activity of ALT in chronic iron toxicity. Furthermore, (28) reported that mild to moderate increases in ALT activity may occur with enzyme induction by drugs and chemicals, including anticonvulsants, corticosteroids, thiacetarsemide and selenium. While (29) concluded that following a single toxic insult, ALT activity increases within (12 hours), peaks within (1-2 days).

- **Serum Cholesterol:** Increase cholesterol agrees with (30, 31, 32, 33, 34). Iron excess induces cellular injury and functional abnormal in hepatocyte by the process of lipid homeostasis. Excess iron may alter the concentration of the serum lipid including total cholesterol (35). The postulated oxidative change in the liver may result in alteration in sterol synthesis leading to increase serum cholesterol levels (36).
- **Serum Glucose:** These results agree with (37, 38, 39, 40). Hyperglycemia commonly occurs early in poisoning. consequently their hepatocytes must rely on such primary energy sources as volatile fatty acids, absorbed from the rumen, rather than, glucose is transported from the intestines or liver to body cells via the bloodstream, and is made available for cell absorption via the hormone insulin, produced by the body primarily in the pancreas (41, 42). So any defects in any one of these lead to alteration of glucose concentration.
- **Iron profile test:** Iron deficiency can be classified into three different stages; storage iron deficiency, iron deficient erythropoiesis and iron deficiency anemia (IDA) (43). Storage iron deficiency characterized by bone marrow iron and serum ferritin are low but serum iron, transferrin saturation, zinc protoporphyrin and complete blood count values are normal, while in iron deficient erythropoiesis bone marrow iron, serum ferritin, serum iron and transferrin saturation are low, zinc protoporphyrin is high but complete blood count values remain within the reference range, on other hand, in IDA bone marrow iron, serum ferritin, serum iron and transferrin saturation are low, zinc protoporphyrin is high and complete blood count values including hemoglobin, MCV, and MCHC are changed (2). Iron over dose could be performed by using many iron compounds in the diet or by injection (44). Iron dextran injected intra peritonealy 100 mg/kg body weight twice weekly for four consecutive weeks could be used (19). Intra peritoneal injection of iron dextran 100mg/day for five days/ week for 8 weeks produced iron toxicity using iron sulfate (45, 46). Iron sucrose (47) and ferrous sulfate (48). or induction of iron toxicity has also been documented. These result Agree with (19, 49, 50). Serum iron increases agree with (51, 52, 53, 54). It has been well documented that the level dietary iron is proportionally related to serum iron (46). Accordingly an increase in serum iron concentration could be due to oral gavages of iron dextran for one month (55). TS% above 70-80% is associated with appearance of labile plasma iron, which is redox active and correlated with generation of ROS and toxicity (56, 57). Many authors prove that iron overdose significantly induced increase in serum iron concentration probably indicating the release of intra cellular rich ferritin into the blood (58). As well as down regulation of iron regulator protein (IRP) activity as common response to increase formation of superoxide anion and H_2O_2 after iron toxicity could be claimed. IRP bind to iron responsive element and stabilize the mRNA for the transferrin receptor while decreasing the translation of mRNA for ferritin (59).

Therefore suspected decreasing in IRP activity after toxicity could increase ferritin synthesis thus enhancing release of iron from liver into circulation with elevation of iron concentration. In the presence of the iron, the iron regularity protein (IRE-BP) detaches from ferritin mRNA, allow in more ferritin to be synthesized. (IRE-BP) serve as different function on transferrin receptor is synthesized (60, 61). A decrease of TIBC during iron toxicity documented by (50, 62). An increase of the TIBC during iron toxicity is documented by (63). Increase of TIBC could reflect a stimulated state of iron absorption as a result of anemia condition. Increase TIBC Agree with (64). while (34) record there are no significant after treatment with iron gluconat.

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