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Amelioration of CCl4 and high fat diet -induced haematological parameters of C57BL/6 mice through lactoferrin

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Abstract
This study was conducted to know the therapeutic efficacy of lactoferrin on haematological parameters in the Non-Alcoholic Fatty Liver Disease (NAFLD) model of male C57BL/6 mice induced by administration of high-fat diet (HFD) + CCl4 (0.5 mg/kg, mixed in olive oil) twice a week via intraperitoneal route for 6 weeks. Thirty-six mice were divided into 6 groups of six animals each. Group 1 served as sham; Group 2 kept as disease Control (HFD + CCl4); Group 3 treated with lactoferrin per se (300 mg/Kg mixed in water) via oral route, Group 4 treated with lactoferrin (300 mg/kg) + HFD + CCl4, Group 5 treated with Lactoferrin (100 mg/kg) + HFD + CCl4 and Group 6 treated with simvastatin (10 mg/kg) + HFD + CCl4.. Blood was collected on the 2nd, 4th and 6th week for the estimation of haematological parameters. The study revealed that lactoferrin dose-dependently prevented elevations in haematological parameters in the NAFLD mice model.

Keywords: NAFLD, high fat diet (HFD), lactoferrin and haemotological parameters

1. Introduction
Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease with a 25% of prevalence rate worldwide [1]. Damage to the hepatocytes and hepatic tissues is due to drugs which lead to NAFLD and prolonged liver diseases [2]. It is estimated that 6% of the adults in developed countries have non-alcoholic steatohepatitis of which 40% of them were advancing towards fibrosis condition [3]. It has two principal phenotypes i.e. nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFLD is associated with increased cardiovascular, cancer and liver-related mortality [4]. Mortality is mainly due to the progression of the disease to cirrhosis, resulting in one-third of deaths worldwide [5, 6].
The C57BL/6 strain in mice, Wistar and Sprague Dawley strain in rats are generally preferred because of their intrinsic predilection to develop obesity, DM2 and NAFLD [7, 8]. Animal models administered with CCl4 and a high-fat diet (HFD) show the features of non-alcoholic steatohepatitis were also based on diet and genetics resulting in liver fibrosis due to oxidative stress and chronic inflammation [9]. CCl4 is an effective hepatotoxin that increases inflammatory response and produces damage to the liver [10].
Simvastatin was used to reduce the elevated liver enzymes and hepatic fatty infiltration in NAFLD patients [11] and to reverse or stabilize fibrosis [12] and also inhibit the HSC proliferation [13] Inhibition of hepatic stellate cells via the Nitric oxide synthase pathway was utilized by simvastatin to ameliorate liver fibrosis [14]
Lactoferrin was first isolated from bovine milk by Sorensen and Sorensen in 1939 [15]. Recently, Lactoferrin has been suggested for potential preventative and adjunct treatment for COVID-19 [16]. Neutrophils containing lactoferrin were released in the blood and inflamed infected tissues [17].
Based on the above facts, the present research was undertaken to study the ameliorative potential of lactoferrin against NAFLD induced alterations in haematological parameters following 6weeks of treatment in male C57BL/6 mice.

2. Materials and Methods
2.1 Chemicals
Carbon Tetra Chloride was procured from M/s Sigma-Aldrich, St. Louis, MO, USA, Lactoferrin was obtained from Bioven Ingredients, India and Simvastatin (SIMVOTIN, 10

mg) from Sun pharmaceutical Ind Ltd, India. Millipore (reverse osmosis) water was employed for oral gavage.

2.2 Experimental animals

The mice strain used for the study was C57BL/6, a classic murine model for experimental NAFLD of 6-7 weeks of age weighing ~28-30g were procured from Vyas Labs, Hyderabad (CPCSEA:2085/PO/Rc.Bi.Bt/S/19/CPCSEA).

This experimental study was approved by the Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, Hyderabad (IAEC, Approval No. CPCSEA 1/24/C.V.Sc, Hyd, IAEC.MICE/ dated 012.06.2021). These animals were kept in polypropylene cages and maintained with 12 hrs dark/light cycle under hygienic conditions having ambient temperature (22–24 ^{0}C) at Animal house in the Department of Veterinary Pharmacology and Toxicology. Animals in control groups 1& 3 were placed on commercial standard pellet feed and animals in the model groups 2, 4, 5 & 6 were fed with a High Fat Diet (M/s. VRK Nutritional solutions, Hyderabad) and provided water ad libitum throughout the experiment

CCl4 treated group than that of control rats (p<0.05), when the CCl4 group treated with a high dose of *Thymus vulgaris* oil significantly (p<0.05) elevated the reduction [23]. The values of RBCs count did not significantly (P>0.05) alter among the treatment groups [26]. There was a significant decrease (P<0.05) in the RBC count, when compared between the CCl4 group and the control group, With respect to the Lactoferrin-protected groups, a significant increase (P<0.05) in the RBC count was observed when compared with the CCl4 group [40] the RBC count results showed no significant difference (P>0.05) between pre- and post-treated in each groups and between post-treatment groups [49]. The interaction of *Withania somnifera* and Vit E could facify the haematological alterations as a results of anti tubercular drugs [51].

Table 1: Total erythrocyte Count (million/µl) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	8.47 ±0.25^a	8.67±0.30^a	8.80±0.26^a
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	6.90±0.13^c	6.78±0.30^c	6.56±0.11^c
3	LF @300mg/Kg p.o	8.54±0.23^a	8.81±0.05^a	8.94±0.19^a
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	7.28±0.21^b	7.47±0.09^b	7.70±0.21^b
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	7.14±0.08^b	7.31±0.19^b	7.50±0.13^b
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P+Standard drug (Simvastatin @ 10mg/Kg p.o)	7.21±0.12^b	7.59±0.14^b	7.90±0.17^b

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly at P<0.05 among the groups.

2.3 Haemoglobin (Hb)

The mean Hb values were significantly (p<0.05) reduced in group 2 (6.78±0.30 and6.56±0.11) when compared with group 1(8.67±0.30 and8.80±0.26) and group 3(8.81±0.05 and 8.94±0.19) on 4th and 6th week of experiment respectively. Significantly (P<0.05) improved values were recorded in group 4(7.47±0.09 &7.70±0.21), group 5(7.31±0.19 & 7.50±0.13) and group6 (7.59±0.14 & 7.90±0.17) when compared with group 2 on 4th and 6th week of experiment respectively. Though, there was no significant difference between groups 4, 5 and 6 on the 4th week. (Table 2.) On the contrary, there was a nonsignificant (P>0.05) decrease in the haemoglobin concentration when CCl4-treated compared with control rats, but reduced haemoglobin concentration was observed in *P.americana* aqueous leaf extract pretreatment group [18]. There was a reduction in the haemoglobin of rabbits that received CCl4 alone when compared to normal control rabbits [19]. The feeding of *withania* to the horses also increases haemoglobin concentration when horses were under stress and rats treated with chlorpyriphos [55, 56]. The current study is in agreement with that of observations on the alcohol extract of *Capparis sepiaria* stem against CCl4 intoxicated Albino rats [29]. Green tea polyphenol extract (600mg/kg) raised the levels of Hb as compared to the CCl4 and high-fat diet-fed model (positive control) [20].

The study revealed that albino rats treated with a high-fat diet and CCl4 showed a decrease in Haemoglobin count [30] there was a decrease in haemoglobin count in rats treated with CCl4 but treatment with miniaturized silymarin & quercetin(MSQ) increased the Hb values which were comparable to the experimental control group [21]. There was a significant reduction (P<0.05) in haemoglobin count in the CCl4-treated group when compared with the control group but treatment with Hepacare significantly (P<0.05) reversed [22]. Haemoglobin was significantly (p<0.05), decreased in the CCl4 group when compared to control rats when the CCl4 group treated with a high dose of *Thymus vulgaris* oil significantly (p<0.05) increased [23]. There was a significant decrease (P<0.05) in the haemoglobin content, when compared between the CCl4 group and the control group, With respect to the Lactoferrin-protected groups, a significant increase (P<0.05) in the haemoglobin content was observed when compared with the CCl4 group [40] Hemoglobin decreased significantly (P<0.05) in the group that received CCl4 and was improved with the administration of oil obtained from seeds of local *Onopordum acanthium L.* [52]

Table 2: Haemoglobin concentration (mg / dL) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	16.47±0.25^a	16.62±0.47^a	16.97±0.36^a
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	13.25±0.21^d	12.91±0.25^c	12.01±0.29^c
3	LF @300mg/Kg p.o	15.98±0.25^b	16.18±0.29^a	16.50±0.24^a
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	15.19±0.25^b	15.31±0.24^b	15.50±0.30^b
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	14.98±0.11^c	15.11±0.18^b	15.60±0.25^b
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P +Standard drug (Simvastatin @ 10mg/Kg p.o)	15.21±0.28^b	15.98±0.24^b	16.12±0.26^a

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly *at* p<0.05 among the groups.

2.4 Platelets count

At 2nd week, there was no significant difference ($P>0.05$) in the platelet count between different groups except group 2. But at 4th & 6th week interval, the platelets count in Group-2 (6.14±0.09 & 5.93±0.24) was significantly ($P<0.05$) lower compared to that of Group1 (8.12±0.14 & 8.45±0.19) and Group 3 (8.17±0.21 & 8.55±0.19). Reported that there was a reduction in Platelets in rabbits that received CCl4 alone, when compared to normal control rabbits [19]. The administration of (*Zizyphus oxyphyla*) at 400 mg/kg body weight significantly reduced the elevated level of platelets when compared to toxic control animals. The current study is in agreement with that of observations on the alcohol extract of *Capparis sepiaria* stem against CCl4 intoxicated Albino rats [29]. Observed that treatment with green tea polyphenol extract (600mg/kg) raised the levels of platelets as compare to the CCl4 and high fat diet fed model (positive control) [20]. Study revealed that albino rats treated with high fat diet and CCl4 showed a decrease in platelets count [30], indicated that there was a decrease in platelets count in rats treated with CCl4 but treatment with miniaturized silymarin & quercetin (MSQ) increased the red blood cell count [21]. Showed significant reduction ($P<0.05$) in platelets count in the CCl4-treated group when compared with the control group, but treatment with Hepacare significantly ($p<0.05$) reversed [22]. Reported that the platelet count results showed no significant difference ($P>0.05$) between pre- and post-treated in each group and between post-treatment groups [49].

Table 3: Total platelet count (Lakhs/cmm) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	7.80±0.08^a	8.12±0.14^a	8.45±0.19^a
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	6.57±0.13^b	6.14±0.09^c	5.93±0.24^c
3	LF @300mg/Kg p.o	7.83±0.18^a	8.17±0.21^a	8.55±0.19^a
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	7.18±0.23^a	7.22±0.11^b	7.33±0.15^b
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	7.07±0.14^a	7.10±0.21^b	7.19±0.17^b
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P +Standard drug (Simvastatin @ 10mg/Kg p.o)	7.12±0.18^a	7.54±0.18^b	7.79±0.12^b

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly *at* $p<0.05$ among the groups.

2.5 Packed cell volume (PCV)

The mean PCV values were significantly ($p<0.05$) reduced in group 2 (43.14±0.33& 42.15±0.37) when compared with group 1(46.14±0.73& 47.56±0.57) and group 3 (46.15±0.16 & 47.23±0.15) on 4th and 6th week of experiment respectively. Significantly ($P<0.05$) improved values were recorded in group 4,5 &6 when compared with group 2 on 4th and 6th week of experiment respectively. Though, there was no significant difference between groups 4, 5 and 6. (Table 4.) On the contrary, there was a nonsignificant ($P>0.05$) decrease in the packed cell volume values when CCl4-treated compared with control rats, but reduced packed cell volume values was observed in *P. americana* aqueous leaf extract pre-treatment group [18]. Significant reduction ($P<0.05$) in packed cell volume in the CCl4-treated group when compared with the control group but treatment with Hepacare significantly ($p<0.05$) reversed [22]. Stated that PCV was significantly ($p<0.05$), decreased in the CCl4 group when compared to control rats when the CCl4 group treated with a high dose of *Thymus vulgaris* oil significantly ($p<0.05$) increased [23]. There was a significant decrease ($P<0.05$) in the PCV values, when compared between the CCl4 group and the control group, With respect to the Lactoferrin-protected groups, significant increase ($P<0.05$) in the PCV values was observed when compared with the CCl4 group [40].

Table 4: PCV (mg / dL) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	45.57±0.76^a	46.14±0.73^a	47.56±0.57^a
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	43.48±0.39^b	43.14±0.33^c	42.15±0.37^c
3	LF @300mg/Kg p.o	45.83±0.43^a	46.15±0.16^a	47.23±0.15^a
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	45.11±0.16^a	45.84±0.18^b	46.83±0.16^b
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	45.05±0.17^a	45.69±0.19^b	46.32±0.11^b
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P+Standard drug (Simvastatin @ 10mg/Kg p.o)	45.27±0.22^a	45.94±0.20^b	46.59±0.15^b

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly *at* $p<0.05$ among the groups.

2.6 Mean corpuscular volume (MCV)

The mean values of MCV were significantly ($P<0.05$) low in group 2 (49.87±0.50 & 48.54±0.55) when compared with group 1(53.88±0.82 & 54.26±0.91) and group 3 (54.93±0.56 & 55.18±1.04) on 4th and 6th week of experiment respectively. Significantly ($P<0.05$) higher values were recorded in group 4,5 &6 when compared with group 2 on 4th and 6th week of experiment respectively. Though, there was no significant difference between groups 4, 5 and 6. (Table 5.) Indicated that there was a decreased mean corpuscular volume (MCV) in rats treated with CCl4 but, groups treated with miniaturized silymarin & quercetin (MSQ) showed increased MCV values which were compared with the experimental control group [21]. Stated that significantly ($p<0.05$) higher values of MCV were observed in CCl4 treated rats than that of the control Meanwhile, the treated CCl4 group with oil of *Thymus vulgaris* changed the values of MCV [23]. Observed that there was no significant ($P>0.05$) change among the treatment groups in MCV values [26]. Reported that there was no significant difference ($P>0.05$) in MCV values when compared between CCl4 treated group and control group [40].

Table 5: MCV (fL) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	53.38±0.74^{b}	53.88±0.82^{b}	54.26±0.91^{b}
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	50.96±0.45^{d}	49.87±0.50^{d}	48.54±0.55^{d}
3	LF @300mg/Kg p.o	54.07±0.39^{a}	54.93±0.56^{a}	55.18±1.04^{a}
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	52.32±0.31^{c}	52.92±0.36^{c}	53.33±0.73^{c}
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	52.00±0.15^{c}	52.53±0.16^{c}	53.18±0.50^{c}
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P +Standard drug (Simvastatin @ 10mg/Kg p.o)	52.03±0.14^{c}	52.91±0.17^{c}	53.56±0.49^{c}

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly *at* $p<0.05$ among the groups.

2.7 Mean corpuscular haemoglobin concentration (MCHC)

The mean values of MCHC were significantly ($P<0.05$) low in group 2 (28.56±1.60&28.02±1.74) when compared with group 1(31.50±0.79&31.87±1.23) and group 3 (31.63±1.06 & 31.99±1.54) on 4th and 6th week of experiment respectively. Though, there was no significant difference between groups other than group 2 on 4th and 6th week. (Table 6.) indicated that there was a decreased mean MCHC count in rats treated with CCl4 but, groups treated with miniaturized silymarin & quercetin (MSQ) showed increased MCHC count which were compared with the experimental control group [21]. Lower MCHC values were observed in CCl4 treated rats than that of the control ($P<0.05$) Meanwhile, the group treated with CCl4 with *Thymus vulgaris* oil changed the values of MCHC [23]. Observed that values of MCHC did not significantly ($P>0.05$) altered among the treatment groups [26]. Reported that there was no significant difference ($P>0.05$) in MCHC values when compared between group teated with CCl4 and control group [40].

Table 6: MCHC (g/dL) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	31.02±0.95^{a}	31.50±0.79^{a}	31.87±1.23^{a}
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	29.15±1.30^{c}	28.56±1.60^{b}	28.02±1.74^{b}
3	LF @300mg/Kg p.o	31.24±1.58^{a}	31.63±1.06^{a}	31.99±1.54^{a}
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	30.09±1.32^{b}	31.33±1.01^{a}	31.42±1.72^{a}
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	30.11±1.40^{b}	31.12±1.30^{a}	31.17±1.58^{a}
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P +Standard drug (Simvastatin @ 10mg/Kg p.o)	30.16±1.30^{b}	31.24±1.15^{a}	31.49±1.21^{a}

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly *at* $p<0.05$ among the groups.

2.8 White blood cell (WBC) count

Significantly ($P<0.05$) increased mean values of WBC were recorded in group 2(9.72±0.19& 10.20±0.19) when compared with group 1(5.63±0.22&5.50±0.18) and group 3(6.22±0.11& 6.13±0.19) on 4th and 6th weeks of experiment. Group 3 recorded significantly ($P<0.05$) high values when compared with groups 1,4,5 & 6 on 4th and 6th week of experiment respectively. There was no significant difference between groups 1, 4, 5 & 6. (Table 7.) there were significantly reduced ($p<0.05$) total white blood cells (WBC) counts when CCl4-treated compared with control rats [18], but increased total white blood cells (WBC) counts was observed in *P. americana* aqueous leaf extract pre-treatment group Administration of CCl4 alone caused leucopenia in the rats similar to the findings of [28]. There was a reduction in the WBC of rabbits that received CCl4 alone, when compared to normal control rabbits [19] The administration of (*Zizyphus oxyphyla*) at 400 mg/kg body weight significantly increased the level of WBC when compared to toxic control animals. The current study is in agreement with observations on the alcohol extract of *Capparis sepiaria* stem against CCl4 intoxicated Albino rats [29]. Green tea polyphenol extract (600mg/kg) decreased the levels of WBC as compare to the CCl4 and high fat diet fed model (positive control) [20]. Study revealed that albino rats treated with high fat diet and CCl4 showed a decrease in WBC count [30], indicated that there was a increase in WBC count in rats treated with CCl4 [21]. WBC was significantly ($p<0.05$), decreased in the CCl4 group when compared to control rats when the CCl4 group treated with a high dose of *Thymus vulgaris* oil significantly ($p<0.05$) increased [23]. Recorded that there was a significant increase ($P<0.05$) in WBC in the CCl4-induced group but not in treated group [23]. Bovine LF supplementation significantly ($P<0.05$) reduced white blood cells (WBC) concentration when compared with control group [26]. There was a significant decrease ($P<0.05$) in the WBC count, when compared between the CCl4 group and the control group, With respect to the Lactoferrin-protected groups, significant increase ($P<0.05$) in the WBC count was observed when compared with the CCl4 group [40]. The WBC count results showed no significant difference ($P>0.05$) between pre- and post-treated in each group and between post-treatment groups [49]. WBC increased significantly ($P<0.05$) in the group that received CCl4 and was improved with administration of oil obtained from seeds of local *Onopordum acanthium* L [52].

Table 7: White blood cell (WBC) count ($x10^3/\mu l$) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	5.91±0.23^{c}	5.63±0.22^{c}	5.50±0.18^{c}
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	7.33±0.24^{a}	9.72±0.19^{a}	10.20±0.19^{a}
3	LF @300mg/Kg p.o	6.84±0.13^{b}	6.22±0.11^{b}	6.13±0.19^{b}
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	6.01±0.21^{b}	5.96±0.10^{c}	5.73±0.16^{c}
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	5.85±0.18^{c}	5.68±0.15^{c}	5.33±0.14^{c}
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P +Standard drug (Simvastatin @ 10mg/Kg p.o)	6.16±0.13^{b}	5.95±0.21^{c}	5.75±0.12^{c}

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly *at* $p<0.05$ among the groups.

2.9 Percent lymphocytes

Mean values of lymphocyte count were significantly ($p<0.05$) reduced in group 2 (50.32±0.23 & 49.63±0.14) when compared with group 1(62.83±0.22 & 63.01±0.19) and group 3(63.61±0.19 & 63.94±0.11) on 4th and 6th week of experiment respectively. Significantly ($P<0.05$) improved values were recorded in group 4,5 &6 when compared with group 2 on 4th and 6th week of experiment respectively. Though, there was no significant difference between groups 1, 3 and 4 on 6th week. (Table 8.) there were significantly increased ($p<0.05$) lymphocytes counts when CCl4-treated compared with control rats [18], but decreased lymphocytes counts was observed in *P. americana* aqueous leaf extract pre-treatment group Administration of CCl4 alone caused lymphocytosis in the rats similar to the findings of [28]. Indicated that there was a decrease in lymphocyte count in rats treated with CCl4 but, treatment with miniaturized silymarin & quercetin (MSQ) increased the lymphocyte number, and the values were comparable to the experimental control group [21]. ymphocytic count was significantly ($p<0.05$), decreased in the CCl4 group when compared to control rats when the CCl4 group treated with a high dose of *Thymus vulgaris* oil significantly ($p<0.05$) increased [23]. Bovine LF supplementation significantly ($P<0.05$ increased lymphocyte count when compared with control group [26]. there was a significant decrease ($P<0.05$) in the lymphocyte counts, when compared between the CCl4 group and the control group, With respect to the Lactoferrin-protected groups, significant increase ($P<0.05$) in the lymphocyte counts was observed when compared with the CCl4 group [40].

Table 8: Lymphocytes count (%) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	62.69±0.19^{b}	62.83±0.22^{b}	63.01±0.19^{a}
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	51.60±0.25^{d}	50.32±0.23^{c}	49.63±0.14^{c}
3	LF @300mg/Kg p.o	63.27±0.09^{a}	63.61±0.19^{a}	63.94±0.11^{a}
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	62.67±0.19^{b}	62.96±0.22^{b}	63.09±0.13^{a}
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	61.67±0.16^{c}	62.01±0.25^{b}	62.33±0.15^{b}
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P +Standard drug (Simvastatin @ 10mg/Kg p.o)	62.38±0.16^{b}	62.69±0.19^{b}	62.98±0.14^{b}

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly *at* $p<0.05$ among the groups.

2.10 Percent Neutrophils

Mean values were significantly ($P<0.05$) increased in the neutrophil count was recorded in group2 (38.20±1.43 & 39.67±1.74) when compared with group 1 (31.44±1.06 & 30.40±1.63) and group 3 (31.67±1.57 &30.96±1.23) on 4th and 6th week of the experiment respectively. Significantly ($P<0.05$) lowered values were recorded in groups 4, 5 & 6 when compared with group 2 on the 4th and 6th week of the experiment respectively. Though, there was no significant difference between groups other than group 2 on the 2nd, 4th and 6th week. (Table 9.) Brai *et al* reported that there were significantly decreased ($p<0.05$) neutrophils when CCl4-treated compared with control rats, but increased neutrophils count was observed in *P. americana* aqueous leaf extract pre-treatment group [18], Administration of CCl4 alone caused neutropenia in the rats similar to the findings of [28]. The study revealed that albino rats treated with a high-fat diet and CCl4 showed a decrease in neutrophils count [30]. There was a significant ($P<0.05$) decrease in neutrophils count when compared with the control group [26]. Reported that normally neutrophils are the first responders to acute inflammation and help in the resolution of inflammation [47]. Reported that G-CSF-deficient mice fed an HFD exhibited a reduction in neutrophil and macrophage infiltration in the liver, alleviating NAFLD progression [48].

Table 9: Neutrophils count (%) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	31.67±1.41^{b}	31.44±1.06^{b}	30.40±1.63^{b}
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	37.67±1.63^{a}	38.20±1.43^{a}	39.67±1.74^{a}
3	LF @300mg/Kg p.o	31.94±1.42^{b}	31.67±1.57^{b}	30.96±1.23^{b}
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	31.77±1.09^{b}	31.38±1.30^{b}	30.63±1.43^{b}
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	31.69±1.57^{b}	31.03±1.20^{b}	30.13±1.98^{b}
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P +Standard drug (Simvastatin @ 10mg/Kg p.o)	31.62±1.44^{b}	31.05±1.09^{b}	30.17±1.37^{b}

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly *at* $p<0.05$ among the groups.

2.11 Percent monocytes

Significantly ($P<0.05$) increased mean values of monocyte count were recorded in group2 (2.73±0.10&3.95±0.05) when compared with group (1.71±0.12&1.83±0.11) and group 3 (1.74±0.21 & 1.95±0.24) on the 4th and 6th week of the experiment respectively. Significantly ($P<0.05$) lowered values were recorded in groups 4, 5 &6 when compared with group 2 on 4th and 6th week of the experiment respectively. Though, there was no significant difference between groups other than group 2 on 2nd, 4th and 6th week. (Table 10.) Observed that the values of monocytes did not significantly ($P>0.05$) change among the treatment groups [26]. There was a significant decrease ($P<0.05$) in the monocyte counts when compared between the CCl4 group and the control group, concerning the Lactoferrin group, a significant increase ($P<0.05$) in the monocyte counts was observed when compared with the CCl4 group [40]. Noticed that monocytes % increased significantly ($P<0.05$) in the group that received CCl4 but reduced with administration of oil obtained from seeds of local *Onopordum acanthium* L [52].

Table 10: Monocytes (%) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	1.65±0.05^{b}	1.71±0.12^{b}	1.83±0.11^{b}
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	2.28±0.08^{a}	2.73±0.10^{a}	3.95±0.05^{a}
3	LF @300mg/Kg p.o	1.68±0.07^{b}	1.74±0.21^{b}	1.95±0.24^{b}
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	1.35±0.25^{b}	1.51±0.09^{b}	1.69±0.26^{b}
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	1.35±0.40^{b}	1.46±0.24^{b}	1.61±0.24^{b}
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P +Standard drug (Simvastatin @ 10mg/Kg p.o)	1.55±0.07^{b}	1.63±0.20^{b}	1.81±0.13^{b}

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly *at* $p<0.05$ among the groups.

2.12 Percent Eosinophils

There was no significant difference in mean values of eosinophil count between groups except group2 (1.86±0.10, 1.98±0.09 &2.08±0.04) on 2nd, 4th and 6th week of experiment respectively the values of Eosinophils did not significantly ($P>0.05$) changed among the treatment groups [26]

Table 11: Eosinophils count (%) in different groups of mice

Group	Treatment	2nd Week	4th Week	6th Week
1	Standard diet	1.64±0.06	1.79±0.05	1.85±0.07^{b}
2	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P	1.86±0.10	1.98±0.09	2.08±0.04^{a}
3	LF @300mg/Kg p.o	1.69±0.07	1.80±0.14	1.88±0.13^{b}
4	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 300mg/Kg p.o)	1.62±0.08	1.75±0.08	1.83±0.07^{b}
5	HFD +CCl4 @ 0.5mg/Kg in olive oil I/P + LF @ 100mg/Kg p.o)	1.61±0.06	1.74±0.04	1.82±0.06^{b}
6	HFD + CCl4 @ 0.5mg/Kg in olive oil I/P +Standard drug (Simvastatin @ 10mg/Kg p.o)	1.61±0.04	1.73±0.09	1.82±0.04^{b}

Mean ± SE (n=6); analysed by One way ANOVA with Duncan's post hoc test (SPSS) Values bearing dissimilar alphabets as superscripts vary significantly *at* $p<0.05$ among the groups.

CCl4 caused a significant reduction in red blood cells and haemoglobin, whereas a significant increase in white blood cells, neutrophils, monocytes, eosinophils and basophils in disease control rats [50]. The aberrations in haematological parameters were normalized by the *Datura stramonium* leaf crude extract in a dose-dependent manner which was identical to silymarin given at a 50 mg/kg dose. There was no significant ($P>0.05$) effect of M. *fragrans* supplementation on MCHC, MCV, and PCV among all groups [53]. studies on the effects of terpenoid and saponin extracts of *I. aquifolium* on lipid metabolism in the Zucker rat model indicate that there was a significant($P<0.05$) increase in RBC, WBC, and platelets in treated groups than control groups [54] CCl4-treated rats displayed a considerable reduction in RBCs count, Hb content, and PCV which could be referred to as the disturbing hematopoiesis, erythrocytes destruction, reducing the erythrocyte formation rate as erythropoiesis is controlled by erythropoietin hormone which is primarily liberated by the kidney and liver [23] CCl4 intoxication induced macrocytic hypochromic anaemia, increased the lipid peroxidation, proteins degradation in the cell membrane, alterations of membrane-bound enzymes, and also increase osmotic fragility of the erythrocyte [31]. Elevation of toxic neutrophils may be imputed to the free radicals liberation during CCl4 metabolism which harmed circulating white blood cell membranes and their content and could also induce several ultra-structural abnormalities in both cytoplasm and nucleus of leukocytes [32] Treating CCl4 intoxicated rats with a high dose of *Thymus vulgaris* essential oil (TEO) significantly elevated total leukocytes and lymphocyte count which could be correlated to the known anti-inflammatory effects of thymol [33]. The release of CCl4 reactive species [trichloromethyl (CCl3.) and trichloromethyl peroxy (CCl3OO.)] might have possibly caused the significant ($P<0.05$) transient reduction in the Haemoglobin concentration and PCV level due to hemolytic anaemia caused by oxidation of sulphydryl groups of the erythrocyte membrane in addition to disturbing hematopoiesis, erythrocytes destruction, rate of formation is reduced and removed from circulation [34, 24]. On the other hand, the CCl4 treatment significantly ($P<0.05$) increased WBCs count which may be attributed to lymphocyte infiltration of poisoned cells, an immune response to a chemical antigen by the body's defensive mechanism of immune system [35, 36, 37]. Meanwhile, treatment with an n-butanol fraction of methanol extract of *F. glumosa* leaves showed significant ($P<0.05$) reversal effects of haematological parameters comparable with normal control rats. The consequent reduction in red blood cells by hemolysis and enhanced hematopoiesis with the decrease in the WBCs count may be ascribed to the stabilization of the free radicals by some antioxidants present in the n-butanol fraction of methanol extract of *F. glumosa* leaves, that was in agreement with the findings of [38, 39]. Aqueous extract of *C. aconitifolius* leaves produced similar action against CCl4-induced hepatotoxicity and haemotoxicity in rats [38].

Normocytic normochromic anaemia was due to MCV and MCHC values where there was no significant difference [40]. Anaemia might be due to the hepatic injury caused by CCl4 and a reduction in the formation of RBC was also observed by [28, 41, 42]. However, the groups that were treated with Lactoferrin showed alleviated changes which were due to an increase in the absorption and utilization of iron as stated by [43]. Lactoferrin loaded with iron may bind to the macrophage surface receptors and deliver its iron to intracellular ferritin stores [44]. The results of the leukogram showed the regulatory role of Lactoferrin in up and down-regulation of the immune system which was mentioned by [45]. The Lactoferrin action may be its ability to stimulate the lymphocyte action [46].

3. Conclusions

The results of this study lead to significant alterations in hematological parameters in male C57BL/6 mice. Hence, Lactoferrin treatment can ameliorate hematological alterations induced by administration of high fat diet (HFD) & CCl4 under experimental conditions.

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6. Conflicts of Interest
The authors declare no conflict of interest

7. References
1. Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, *et al*. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. Hepatology. 2019;69:2672-2682.
2. Yu Y, Cai J, She Z, Li H. Insights into the Epidemiology, Pathogenesis, and Therapeutics of Nonalcoholic Fatty Liver Diseases. Adv. Sci. 2019;6:1801585.
3. Abeysekera KW, Fernandes GS, Hammerton G, Portal AJ, Gordon FH, Heron J. Prevalence of steatosis and fibrosis in young adults in the UK: a population-based study. Lancet Gastroenterol Hepatol. 2020;5:295-305. https://doi.org/10.1016/S2468-1253(19)30419-4.
4. Wattacheril J, Chalasani N. Non-Alcoholic Fatty Liver Disease (NAFLD): Is it really a serious condition? Hepatology. 2012 Oct;56(4):1580-1584.
5. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016 Jul;64(1):73–84.
6. Higashi T, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis. Adv Drug Deliv Rev. 2017;121:27-42. https://doi.org/10.1016/j.addr.2017.05.007.
7. Kohli R, Feldstein AE. Nash animal models: Are we there yet? J Hepatol. 2011;55:941-943.
8. Takahashi Y. Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol. 2012;18:2300-2308.
9. Kubota N, Kado S, Kano M, Masuoka N, Nagata Y, Kobayashi T, Miyazaki K, *et al*. A high-fat diet and multiple administration of carbon tetrachloride induces liver injury and pathological features associated with non-alcoholic steatohepatitis in mice. Clin Exp Pharmacol Physiol. 2013;40:422-430.
10. Ostovaneh MR, Ambale-Venkatesh B, Fuji T, Bakhshi H, Shah R, Murthy VL. Association of Liver Fibrosis with Cardiovascular Diseases in the General Population: The Multi-Ethnic Study of Atherosclerosis (MESA). Circ. Cardiovasc. Imaging. 2018;11,No:e007241.
11. Abel T, Fehér J, Dinya E, Eldin MG. Kovács A Safety and efficacy of combined ezetimibe/simvastatin treatment and simvastatin monotherapy in patients with non-alcoholic fatty liver disease. Med Sci. Monit. 2009;5:MS6-11: MS6-11
12. Ekstedt M, Franzén LE, Mathiesen UL, Holmqvist M, Bodemar G, *et al*. Statins in nonalcoholic fatty liver disease and chronically elevated liver enzymes: a histopathological followup study. J Hepatol. 2000;47:135-141.
13. Rombouts K, Kisanga E, Hellemans K, Wielant A, Schuppan D, *et al*. Effect of HMG-CoA reductase inhibitors on proliferation and protein synthesis by rat hepatic stellate cells. J Hepatol. 2003;38:564-572.
14. Wang W, Zhao C, Zhou J, Zhen Z, Wang Y, Shen C. Simvastatin ameliorates liver fibrosis via mediating nitric oxide synthase in rats with non-alcoholic steatohepatitis-related liver fibrosis. PLoS One. 2013;8:e76538
15. Ahmed Kazi Ahsan, Abu Saim Mohammad Saikat, Akhi Moni, Sadia Akhter Mallik Kakon, Md. Rashedul Islam, Md Jamal Uddin. Lactoferrin: potential functions, pharmacological insights, and therapeutic promises. J Adv Biotechnol Exp Ther. 2021 May;4(2):223-237
16. Chang R, Ng TB, Sun WZ. Lactoferrin as potential preventative and adjunct treatment for COVID-19. Int J Antimicrob Agents. 2020;56:106118.
17. Farnaud S, Evans RW. Lactoferrin-a multifunctional protein with antimicrobial properties. Molecular Immunology,. 2003;40(7):395-405.
18. Brai BIC, Falode JA, Adisa RA, Odetola A. A Effects of aqueous leaf extract of avocado (*Persea americana*) on total cholesterol, triacylglycerols, protein and haematological parameters in CCl4-intoxicated rats. Brai *et al.* Clinical Phytoscience. 2020;6:14. https://doi.org/10.1186/s40816-020-00159-y
19. Ahmad B, Ilahia I, Yousafzaib AM, Attaullaha M, Rahima A. Protective effects of *Zizyphus oxyphyla* on liver and kidney related serum biomarkers in (CCl4) intoxicate rabbits. Brazilian Journal of Biology. 2023;83:e246980. https://doi.org/10.1590/1519-6984.246980
20. Kanwal HA, Majeed W, Awan AM, Aslam B. Polyphenols from Green tea revert CCl4-Induced liver oxidative stress and inflammation associated with high fat diet by activation of NRF-2 and TIMP-1pathways to neutralize ROS. Research square, 2022. DOI: https://doi.org/10.21203/rs.3.rs-1414824/v1
21. Jaisheela MSR, Muthukumar SP, Srivastava AK. Effect of Silymarin and Quercetin in a Miniaturized Scaffold in Wistar Rats against Non-alcoholic Fatty Liver Disease. ACS Omega. 2021;6:20735-20745. https://doi.org/10.1021/acsomega.1c00555.
22. Adebayo AH, Yakubu OF, Adegbite OS, Okubena O. Haematopoietic induction and hepatic protective roles of Hepacare® in CCl4-induced hepatic damaged rats. Comp Clin Pathol, 2017. DOI 10.1007/s00580-017-2428-0
23. Elshopakey GE, Risha EF, El-Boshy ME, Abdalla OA, Hamed MF. Protective effects of *Thymus vulgaris* oil against CCl4-mediated hepatotoxicity, oxidative stress and immunosuppression in male albino rats. *Adv.* Anim. Vet. Sci. 2021;9(7):1053-106 http://dx.doi.org/10.17582/journal.aavs/2021/9.7.1053.1063.
24. Abu MS, Yakubu OCE, Onuche JI, Okpe O. Lipid profiles, hematological parameters and histopathological analysis of CCl4-intoxicated wistar albino rats treated with n-butanol extract of *Ficus glumosa* leaves. Cell Biology & Development. 2022;6:6-12. DOI: 10.13057/cellbioldev/t060102.
25. Sirichaiwetchakoon K, Lowe GM, Kupittayanant S, Churproong S, Eumkeb G. *Pluchea indica* (L.) Less. Tea Ameliorates Hyperglycemia Dyslipidemia, and Obesity in High Fat Diet-Fed Mice. Hindawi Evidence-Based Complementary and Alternative Medicine. 2020, 12. doi.org/10.1155/2020/8746137.
26. Mallaki M, Hosseinkhani A, Taghizadeh A, Hamidian G,

Paya H. The Effect of Bovine Lactoferrin and Probiotic on Performance and Health Status of Ghezel Lambs in Preweaning Phase. Iranian Journal of Applied Animal Science. 2021;11(1):101-110.
27. Ekor M, Owusu Agyei PE, Obese E, Biney RP, Henneh IT, Antwi-Adjei M, *et al*. Celecoxib exhibits therapeutic potential in experimental model of hyperlipidaemia. PLoS ONE. 2021;16(8):e0247735. https://doi.org/10.1371/journal. pone.0247735
28. Mandal A, Karmakar R, Bandyopadhyay S, Chatterjee M. Antihepatotoxic potential of *Trianthema portulacastrum* in carbon tetrachloride-induced chronic hepatocellular injury in mice: reflection in haematological, histological and biochemical characteristics. Arch Pharm Res. 1998;21:223. https://doi.org/10.1007/BF02975279.
29. Cordeiro M, Kaliwal B. Protective role of bark extract of *Bridelia retusa* Spreng on CCl4 induced histological toxicity in mice. Journal of Pharmacognosy and Phytochemistry. 2013;2(4):142-148.
30. Wang D, Wang Y, Madhu S, Liang H, Bray CL. Total hemoglobin count has significant impact on A1C—Data from National Health and Nutrition Examination Survey 1999–2014. Prim Care. 2019;13:316-323. https://doi.org/10.1016/j.pcd.2019.01.002.
31. Meral I, Kanter M. Effects of Nigella sativa L. and Urtica dioica L. on Selected Mineral Status and Hematological Values in CCl4-Treated Rats. Biolog. Trace Element Res. 2003;96:263-270. https://doi.org/10.1385/BTER:96:1-3:263
32. Makni M, Chtourou Y, Fetoui H, Garoui EM, Barkallah M, Marouani C. Erythrocyte oxidative damage in rat treated with CCl4: protective role of vanillin. Toxicol. Indus. Health. 2012;28:908-916. https://doi. org/10.1177/0748233711427055.
33. Sinha AK, Rijal S, Karki P, Majhi S. Incidence of megaloblastic anaemia and its correction in leishmaniasis: A prospective study at BPKIHS hospital, Nepal. Indian J Pathol. Microbiol. 2006;49:528-531.
34. Chan AS, Pang H, Yip ECH, Tam YK, Wong YH. Carvacrol and Eugenol Differentially Stimulate Intracellular Ca2+Mobilization and Mitogen-Activated Protein Kinases in Jurkat T-Cells and Monocytic THP-1 Cells. Planta Med. 2005;71:634-639. https://doi.org/10.1055/s-2005-871269.
35. Khalid GA, Fathia AM, Mosaad AA. The protective effects of whey protein and spirulina against CCl4-induced erythrocyte damage in rats. J Appli Sci. Res. 2013;9(3):2063-2071.
36. Maduka HC, Daja A, Okoye Gadaka GA, Abubakar KA, Maduka AA. Protective role of *Moringa oleifera* Lam aqueous extract on some excretory products and hematological parameters in acetamophen induced albino rats. J Nutr Health. 2014;3(2) 27-31. DOI: 10.9790/1959-03252731
37. Mariam GE, Hassenane MM, Ibrahim MF, Nermeen MS, Abodfetoh MA. Evaluation of protective and therapeutic role of moringa oleifera leaf extract on CCL4-induced genotoxicity, haematoxicity and hepatotoxicity in rats. Intl J Pharm Tech Res. 2015;7(2):392-415.
38. Saba AB, Oyagbemi AA, Azeez OI. Amelioration of CCl4-induced hepatotoxicity and haematotoxicity by aqueous leaf extract of *Cnidoscolus aconitifolius* in rats. Niger J Physiol Sci. 2010;25:139-147.
39. Yakubu OE, Ojogbane EB, Abu MS, Shaibu CO, Ayegba WE. Haematinic effects of ethanol extract of *Ficus sur* leaves on diethylnitrosamine-induced toxicity in wistar rats. J Pharm Toxicol. 2020;15(1):16-21. DOI: 10.3923/jpt.2020.16.21.
40. Farid AS, Mona A, Shemy El Nafie E, Hegazy AM, Abdelhiee EY. Anti-inflammatory, anti-oxidant and hepatoprotective effects of lactoferrin in rats, Drug and Chemical Toxicology. 2019;44:NO.3,286-293. DOI: 10.1080/01480545.2019.1585868
41. Essawy AE, Ashraf M, Moneim A, Khayyat LI, Elzergy AA. Role of black seeds (*Nigella sativa*) in ameliorating carbon tetrachloride-induced haematotoxicity in Swiss albino mice. Journal of Medicinal Plants Research. 2010;4(19):1977-1986. DOI: 10.7324/JAPS.2012.21004
42. Amer MA, EL-missiry MA, EL-nabi AA. The role of *Ficus carica* leaf extract in modulation of the experimentally induced hepatotoxic damage in male rats. International Journal of Advanced Research. 2015;3(12):572-585.
43. Koikawa N, Nagaoka I, Yamaguchi M, Hamano H, Yamauchi K, Sawaki K. Preventive effect of lactoferrin intake on anemia in female long-distance runners. Bioscience, Biotechnology, and Biochemistry. 2008;72(4):931-935. DOI: 10.1271/bbb.70383
44. Birgens HS. The biological significance of lactoferrin in haematology. Scandinavian Journal of Haematology. 1984;33(3):225-230.
45. Ward PP, Conneely OM. Lactoferrin: Role in iron homeostasis and host defense against microbial infection. Biometals: An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine. 2004;17(3):203-208.
46. Zimecki M, Stepniak D, Szynol A, Kruzel ML. Lactoferrin regulates proliferative response of human peripheral blood mononuclear cells to phytohemagglutinin and mixed lymphocyte reaction. Archivum Immunologiae Et Therapiae Experimentalis-English Edition. 2001;49(2):147-154.
47. Cervera AH, Soehnlein O, Kenne E. Neutrophils in chronic inflammatory diseases. Cellular & Molecular Immunology. 2022;19:177-191; https://doi.org/10.1038/s41423-021-00832-3.
48. Zhang Y, Zhou X, Liu P, Chen X, Zhang J, Zhang H, *et al*. GCSF deficiency attenuates nonalcoholic fatty liver disease through regulating GCSFR-SOCS3-JAK-STAT3 pathway and immune cells infiltration. Am J Physiol Liver Physiol. 2021;320:G531–42.
49. Hengpratom T, Kupittayanant S, Churproong S, Eumkeb G. Lipid-lowering effect of *Oroxylum indicum* (L.) Kurz extract in hyperlipidemic mice. Asian Pac J Trop Biomed. 2022;12(4):148-155.
50. Nasir B, Khan AU, Baig MW, Althobaiti YS, Faheem M, Ihsan-Ul Haq. *Datura stramonium* Leaf Extract Exhibits Anti-inflammatory Activity in CCL4-Induced Hepatic Injury Model by Modulating Oxidative Stress Markers and iNOS/Nrf2 Expression. BioMed Research International 2022, Article ID 1382878, 20 pages. https://doi.org/10.1155/2022/1382878
51. Arundhathi S, Anand Kumar A, Ravi Kumar Y, Anil Kumar B. Haematological and histopathological alterations due to combined toxicity of Isoniazid and Rifampicin; amelioration with *Withania somnifera* and Vitamin-E in Wistar rats. International Journal of Pharma

and Bio Sciences. 2015;6:P222-P229.
52. Muhammad HA, Najeeb HA, Saleh Jubrail AM. Physiological and Immunological Study for the Effects of *Onopordum acanthium* L. Seeds Oil in Male Rats Treated with CCL4. Iraqi Journal of Science. 2022;63(5):1918-1929. DOI: 10.24996/ijs.2022.63.5.6.
53. Rashidian G, Shahin K, Elshopakey GE, Mahboub HH, Fahim A, Elabd H. The Dietary Effects of Nutmeg (*Myristica fragrans*) Extracton Growth, Hematological Parameters, Immunity, Antioxidant Status, and Disease Resistance of Common Carp (*Cyprinus carpio*) against *Aeromonas hydrophila*. J Mar. Sci. Eng. 2022;10:325. https://doi.org/10.3390/jmse10030325.
54. Pachura N, Kupczy´ Nski R, Lewandowska K, Włodarczyk M, Klemens M, Kuropka P, *et al*. A Biochemical and Molecular Investigation of the Effect of Saponins and Terpenoids Derived from Leaves of *Ilex aquifolium* on Lipid Metabolism of Obese Zucker Rats. Molecules. 2022;27:3376. https://doi.org/10.3390/molecules27113376.
55. Manvitha Vellanki A, Gopala Reddy B, Anil Kumar M, Jeevanalatha, Priyanka G. Protective Role of Ashwagandha and Selenium against Chlorpyrifos (CPF) Induced Haemato-Biochemical and Hepatic Alterations in Wistar Rats. Int. J Curr. Microbiol. App. Sci. 2019;8(11):941-949.
56. Priyanka G, Anil Kumar B, Lakshman M, Manvitha V, Kala Kumar B. Adaptogenic and immunomodulatory activity of Ashwagandha root extract: An experimental study in an Equine model. Frontiers in veterinary science 2020, 700.