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Insights into hematological alterations in sepsis in pre-existing diabetes in murine model

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Abstract
The present research aimed to explore the impact of sepsis on hematological changes in mice with pre-existing diabetes. Type 1 diabetes was induced by administering streptozotocin at 65 mg/kg body weight via intraperitoneal route for five consecutive days. Sepsis was induced through caecal ligation and puncture. To create a diabeto-septic condition, sepsis was induced after confirming diabetic conditions (8 weeks after the first streptozotocin dose). Blood samples were collected from all groups for hematological parameter assessment. Sepsis led to a significant increase in hemoglobin levels, accompanied by a decrease in other hematological indices, including total erythrocyte count, total leukocyte count, platelet count, hematocrit, granulocytes, monocytes, and lymphocytes. Diabetic mice showed significant reduction in total leukocyte count and lymphocytes, an increase in granulocytes and platelets, with no significant changes in other hematological indices compared to the healthy control. In diabeto-septic mice, hemoglobin levels, total erythrocyte counts, and hematocrit values were nearly comparable to the healthy control, sham-operated, and diabetes groups. However, total leukocyte count and lymphocytes values were found to be intermediate between diabetes and septic mice, but not significantly different from these groups in sepsis in pre-existing diabetic mice. Additionally, monocyte count was significantly lower in diabeto-sepsis than in diabetes, showing significant higher level from sepsis. Granulocytes were significantly decreased in diabeto-sepsis compared to diabetes and were almost comparable to sepsis. Interestingly, sepsis in pre-existing diabetes decreased platelet count to almost normal levels. In conclusion, the coexistence of sepsis and diabetes appears to alter hematological alterations caused by these two conditions individually. However, further studies are necessary to comprehensively understand these interactions.

Keywords: Sepsis, diabetes, hematological, diabeto-septic

Introduction
Sepsis is a critical dysfunction of organs that poses a life-threatening risk and is triggered by an imbalanced response from the body to infection. There were around 49 million reported cases of sepsis globally in 2017, leading to approximately 11 million deaths (Rudd *et al.*, 2020; Guarino *et al.*, 2023) [1, 2]. Diabetes mellitus is a metabolic syndrome characterized by defective control over plasma glucose either due to insulin deficiency or insulin resistance (Watkins and Sanders, 1995) [3]. Over the past few decades, there has been a rising prevalence of diabetes mellitus. Diabetes and sepsis play significant roles in the worldwide burden of illness and mortality. Individuals with diabetes, identified as the largest at-risk for elevated probabilities of complications after sepsis and an increasing mortality rate (Costantini *et al.*, 2021) [4]. The presence of diabetes also heightens the likelihood of various types of infections that can result in sepsis (Schuetz *et al.*, 2011; Sun *et al.*, 2023) [5, 6]. The hematologic system of body is affected in septic condition. The early signs of severe sepsis often involve dysfunction in hematologic system, a manifestation observed in nearly all patients affected by this condition. The malfunction of hematologic system may play a role in the development of multiple organ dysfunctions and can lead to fatal outcomes (Goyette *et al.*, 2004) [7]. Furthermore, alterations in hematology have also been documented in diabetes and contribute significantly to the complications associated with the condition (Arkew *et al.*, 2021) [8]. Therefore, the present study was undertaken to explore the effect of sepsis on the hematological parameters in pre-existing diabetes condition in mouse model.

Materials and methods

Experimental animals

Adult male Swiss albino mice (26-28 g) were procured from Disease Free Small Animal House, College of Veterinary and Animal Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana. Before starting experiments, animals were acclimatized for 10 days by housing in polypropylene cages in the Departmental Laboratory Animal House under 12-12 h dark-light cycle along with *ad libitum* feed and water. The study was undertaken after approval from the Institutional Animal Ethics Committee (IAEC) of the University (Approval No.: IAEC/17/12, Dated 26-07-2017).

Induction of type-1 diabetes

Streptozotocin (Sigma-Aldrich) was used to induce the type^{-1} diabetes. Streptozotocin solution was prepared in 0.1M citrate buffer (pH 4.5) and injected immediately for five consecutive days at 65 mg/body weight (intraperitoneally) as described by Furman (2015) [9] with some modifications. Blood glucose level was measured weekly and animals showing >300 mg/dl of blood glucose level were considered as diabetic.

Induction of sepsis

The caecal ligation and puncture (CLP) method described earlier (Wichterman *et al.*, 1980; Mishra and Choudhury, 2018) [10, 11] was used to induce sepsis. Following overnight fasting, mice were anesthetized, and a midline incision was made to expose the cecum, which was then ligated and punctured twice with a 21G needle. Sham-operated (SO) mice underwent a similar surgical procedure without CLP as controls. Isotonic sodium chloride solution was administered to prevent dehydration. After surgery, mice were monitored for sepsis development over 72 hours, assessing lethargy, conjunctivitis, grooming behavior, fur condition, and feed and water intake.

Induction of diabeto-sepsis

In diabeto-septic group, firstly diabetes was induced by administering STZ. After 8 weeks of the 1st dose of streptozotocin, diabetic condition in mice was confirmed and sepsis was induced by CLP method.

Collection of samples

18 h post-surgery i.e. during late phase of sepsis, 18h after surgery in sham-operated, eight weeks after 1st dose of citrate buffer and streptozotocin administration from healthy control and diabetes mice, 13 h post-surgery in diabeto-sepsis co-existing condition, blood samples were collected through cardiac puncture in EDTA containing tubes and haematological parameters were estimated by auto-analyser (Mindray).

Results

a) Effect on Haemoglobin level

There were no significant differences in the haemoglobin levels between the mice of healthy control (11.67±0.38 g/dl; n=6), sham-operated (11.45±0.45 g/dl; n=6), diabetes (12.10±0.93 g/dl; n=6) and diabeto-septic (13.07± 0.21 g/dl; n=6) groups. But haemoglobin level was found to be significantly (p<0.001) higher in mice of sepsis group (15.92±0.98 g/dl; n=6) compared to the mice of sham-operated and all other groups. Furthermore, the haemoglobin level in diabeto-septic mice (13.07± 0.21 g/dl; n=6) was significantly (p<0.05) decreased compared to the sepsis group (15.92±0.98 g/dl; n=6) (Figure 1).

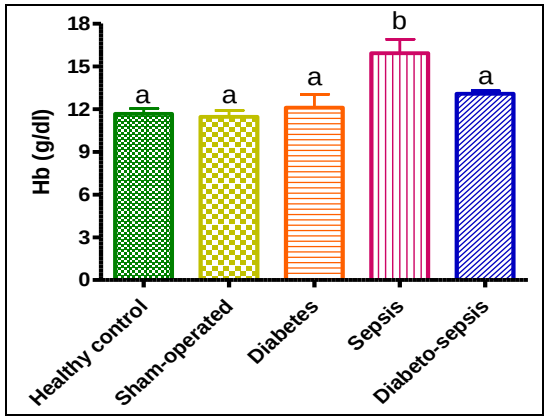

Fig 1: Bar diagrams showing the effect of diabetes, sepsis and co-existing diabetes and sepsis on the haemoglobin levels in mice of different groups. Data were analyzed by one-way ANOVA followed by Tukey's *post-hoc* test. Different superscripts on the bars indicate significant (p<0.05-0.001) differences between different groups

b) Effect on total erythrocytes count (TEC)

The values of total erythrocyte count (TEC) in the mice of healthy control (10.39±0.63×10^6/µl; n=6), sham-operated (10.37±0.74 ×10^6/µl; n=6), diabetes (10.48±0.98×10^6/µl; n=6) and diabeto-septic (9.33±0.26×10^6/µl; n=6) groups were almost comparable and no significant difference was observed among these groups. However, the values of TEC in mice of the sepsis group (6.28±0.59 ×10^6/µl; n=6) was found to be significantly (p<0.01) lower compared to the mice of sham-operated (10.37±0.74 ×10^6/µl; n=6). Furthermore, a significantly (p<0.05) higher level of TEC was noted in diabeto-septic mice (9.33±0.26×10^6/µl; n=6) than sepsis group (6.28±0.59 ×10^6/µl; n=6) (Figure 2).

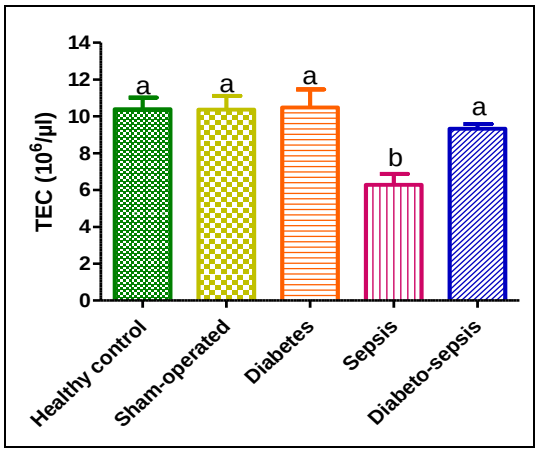

Fig 2: Bar diagrams showing the effect of diabetes, sepsis and co-existing diabetes and sepsis on the total erythrocytes count (TEC) in mice of different groups. Data were analyzed by one-way ANOVA followed by Tukey's *post-hoc* test. Different superscripts on the bars indicate significant (p<0.05-0.01) differences between the different groups

c) Effect on total leucocytes count (TLC)

No significant alterations in the values of TLC between the mice of healthy control (12.38±0.64 ×10^3/µl; n=6) and sham-operated (12.52±0.65 ×10^3/µl; n=6) groups. However, the values of TLC in mice of diabetes (5.93±0.95 ×10^3/µl; n=6) and sepsis groups (2.02±0.10 ×10^3/µl; n=6) were significantly (p<0.001) lower compared to the mice of healthy control and

sham-operated groups. But the values of TLC in diabeto-septic group (3.85±0.78×10^3/µl; n=6) did not differ significantly from the values of TLC in mice of diabetes and sepsis groups (Figure 3).

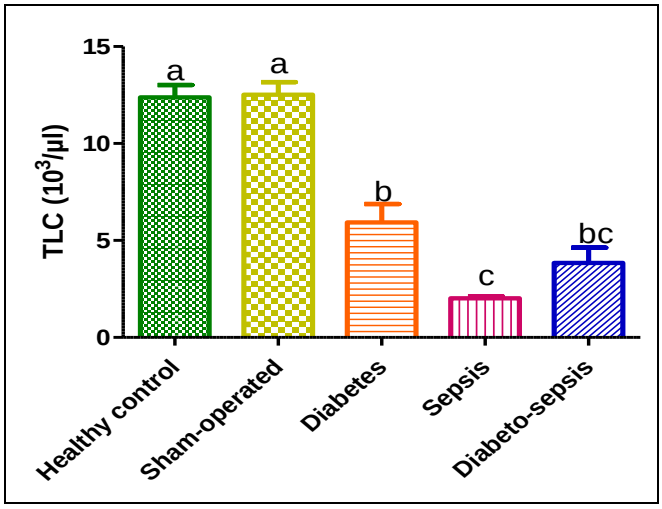

Fig 3: Bar diagrams showing the effect of diabetes, sepsis and co-existing diabetes and sepsis on the total leucocytes count in mice of different groups. Data were analyzed by one-way ANOVA followed by Tukey's *post-hoc* test. Different superscripts on the bars represent the significant (p<0.001) differences between different groups

d) Effect on absolute differential leucocyte count (lymphocyte, monocyte and granulocyte)

The absolute value of lymphocytes counts in mice of healthy control (7.60±0.85 ×10^3/µl; n=6) and sham-operated (7.68±0.89 ×10^3/µl; n=6) groups did not significantly differ from each other. However, the absolute count of lymphocytes in mice of diabetes (4.65±0.81 ×10^3/µl; n=6) was significantly (p<0.05) reduced compared to healthy control (7.60±0.85 ×10^3/µl; n=6). Furthermore, a significant (p<0.001) decrease in lymphocyte was also observed in sepsis (1.33±0.14 ×10^3/µl; n=6) groups compared to those in mice of sham-operated groups (7.68±0.89 ×10^3/µl; n=6). But lymphocytes count in mice of diabeto-septic group (2.75±0.48 ×10^3/µl; n=6) did not significantly differ from those observed in mice of diabetes and sepsis groups; although the values in diabeto-sepsis group were significantly lower than in mice of healthy control and sham-operated groups (Figure 4).

A significant (p<0.001) reduction in absolute monocyte count was observed in sepsis group (0.08±0.02×10^3/µl; n=6) compared to that in sham-operated animals (0.45±0.04 ×10^3/µl; n=6). In case of diabeto-septic mice group (0.27±0.06×10^3/µl; n=6) also, significant (p<0.01) decrease in monocytes count was observed compared to diabetes group; but significantly (p<0.05) higher than in mice of septic mice. Absolute monocytes count in mice of healthy control (0.48±0.04 ×10^3/µl; n=6), sham-operated (0.45±0.04 ×10^3/µl; n=6) and diabetes (0.50±0.05 ×10^3/µl; n=6) groups did not significantly differ from each other (Figure 4).

No significant changes in the values of granulocytes were observed between the mice of healthy control (3.65±0.18×10^3/µl; n=6) and sham-operated (3.75±0.20×10^3/µl; n=6) groups. However, compared to both these groups, significant (p<0.05) increase in the values of granulocytes in mice of diabetes groups (5.62±0.81×10^3/µl; n=6) was observed. Contrary to the diabetes group, significantly (p<0.001) lower count of granulocytes was observed in mice of sepsis (0.53±0.08 ×10^3/µl; n=6) and diabeto-sepsis group (1.43±0.24 ×10^3/µl; n=6). Furthermore, a significant (p<0.001) decrease in granulocytes was seen in diabeto-septic mice (1.43±0.24 ×10^3/µl; n=6) compared to diabetes group (5.62±0.81×10^3/µl; n=6). The values of granulocytes between sepsis and diabeto-sepsis groups did not differ significantly (Figure 4).

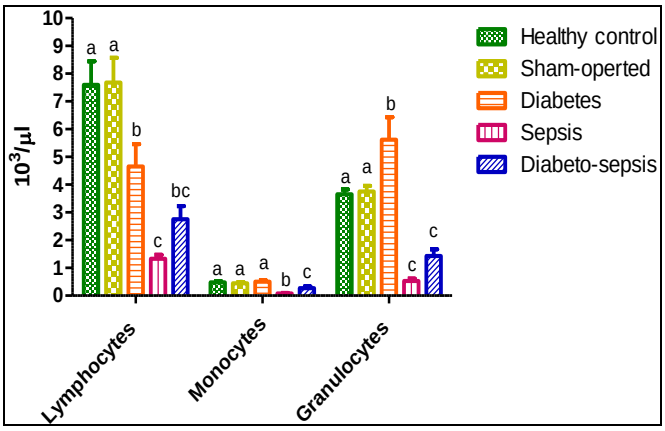

Fig 4: Bar diagrams showing the effect of diabetes, sepsis and co-existing diabetes and sepsis on the absolute values of differential leucocytes count (Lymphocytes, Monocytes and Granulocytes) in mice of different groups. Data were analyzed by one-way ANOVA followed by Tukey's *post-hoc* test. Different superscripts on bars represent significant (p<0.05-0.001) differences between the groups

e) Effect on platelets count

A significant (p<0.01) increase in platelets count in diabetes (2052±171.1 ×10^3/µl; n=6) group, while significant (p<0.01) decrease in mice of sepsis group (711.7±68.39 ×10^3/µl; n=6) was observed compared to healthy control (1377±127.3 ×10^3/µl; n=6) and sham-operated groups (1373±123.5 ×10^3/µl; n=6). There was no significant difference seen between the platelets count in mice of healthy control (1377±127.3 ×10^3/µl; n=6) and sham-operated (1373±123.5 ×10^3/µl; n=6) groups. But interestingly, in the mice of diabeto-septic group, platelets count (1409±60.04×10^3/µl; n=6) was significantly (p<0.01) higher than in the mice of sepsis group comparable to that in mice of healthy control and SO group. Although, it was significantly (p<0.01) lower compared to that in the diabetes group (Figure 5).

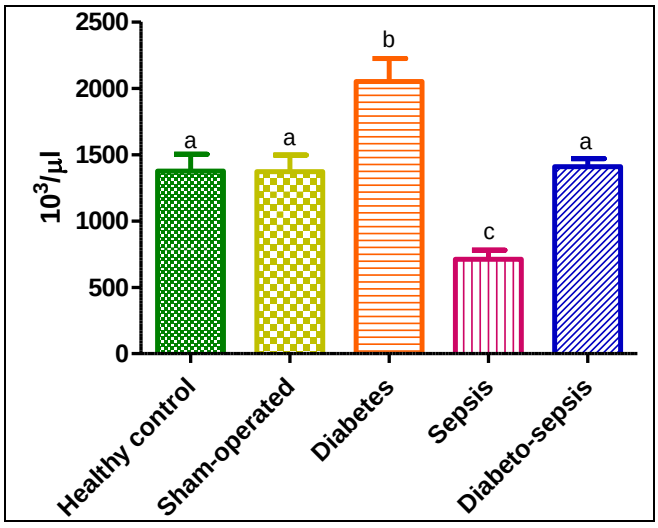

Fig 5: Bar diagrams showing the effect of diabetes, sepsis and co-existing diabetes and sepsis on platelets count in mice of different groups. Data were analyzed by one-way ANOVA followed by Tukey's *post-hoc* test. Different superscripts on bars represent significant (p<0.01) differences between the mice of different groups

f) Effect on haematocrit (HCT)

The HCT value in sepsis group (34.10±1.37%; n=6) was significantly (p<0.01) lower compared to the sham-operated (48.77±2.84%; n=6). However, the HCT values in mice of healthy control (48.73±2.38%; n=6), sham-operated (48.77±2.84%; n=6), diabetes (51.65±4.24%; n=6) and diabeto-sepsis (46.97±1.74%; n=6) groups were almost comparable to each other. Furthermore, a significant (p<0.05) increase in HCT was noted in diabeto-septic group (46.97±1.74%; n=6) compared to sepsis group (34.10±1.37%; n=6) (Figure 6).

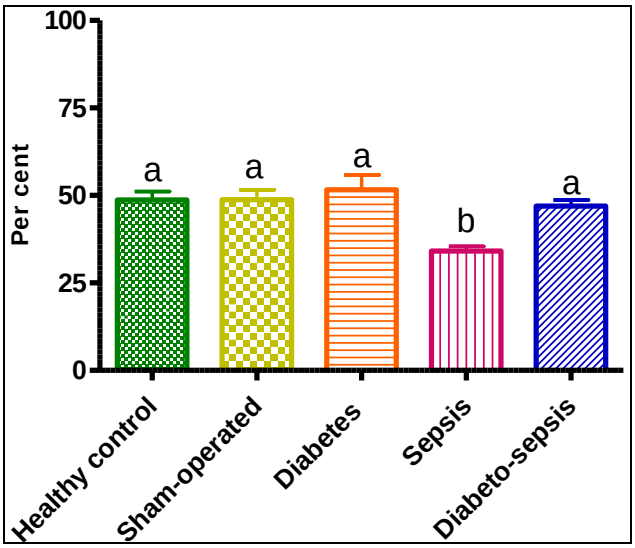

Fig 6: Bar diagrams showing effect of diabetes, sepsis and co-existing diabetes and sepsis on haematocrit levels in the mice of different groups. Data were analyzed by one-way ANOVA followed by Tukey's *post-hoc* test. Different superscripts on bars represent significant (p<0.05-0.01) differences between the mice of different groups

Discussion

Septic patients are mostly reported to have with decreased haemoglobin levels, leukocytosis, anaemia, thrombocytopenia, and decrease in the haematocrit value (Aird, 2003) [12]. In the present study, we observed decrease in RBC, WBC, granulocytes, platelets count, hematocrits and increased hemoglobin level. These findings are concordant to previous study which showed the protective effect of the erythropoietin on clinical severity and hematological changes in septic mice (Kannan *et al.*, 2020) [13]. Sepsis causes haemolysis due to destruction of RBC membrane by reactive oxygen species, which leads to decrease in RBC count and increase in free-haemoglobin (Bateman *et al.*, 2001; Aird, 2003) [14, 12]. Furthermore, in our study decrease in lymphocyte and monocyte level in sepsis was seen, which are in agreement with earlier reports (Drewry *et al.*, 2014; Sáenz *et al.*, 2001; Chung *et al.*, 2019) [15, 16, 17]. We found no significant difference in haemoglobin, TEC count and monocytes, significant increase in TLC, granulocytes, and platelets and significant decrease in lymphocytes in mice of diabetes group which are in accordance with the findings of earlier researchers (Sterner *et al.*, 1998; Akah *et al.*, 2009; Mahmoud *et al.*, 2013) [18, 19, 20]. Sterner and co-workers (1998) [18] have suggested that raised platelets count in type 1 diabetes could be used as a prognostic indicator of future complications especially diabetic-nephropathy. In diabeto-sepsis group, no significant difference was observed in haemoglobin, TEC level as compared to diabetes group, but these values were found to be significantly higher than in sepsis group, whereas TLC and lymphocytes values did not differ from that in diabetes and sepsis groups. Monocytes were found to be significantly higher than in sepsis, but significantly lower than in diabetes group. Granulocytes in diabeto-sepsis group increased but not differ significantly than in sepsis group, while significantly lower than in diabetes group. Interestingly, sepsis in pre-existing diabetes shifted the platelet count to normal level which was almost comparable to that in healthy control group.

Conclusion

Sepsis induces changes in hematological parameters, including an increase in hemoglobin and a decrease in total erythrocyte count (TEC), total leukocyte count (TLC), lymphocytes, monocytes, granulocytes, platelet count, and hematocrit (HCT). Additionally, diabetic mice exhibited a reduction in TLC and lymphocytes, along with an increase in granulocytes and platelets. In the context of pre-existing diabetes, sepsis resulted in hemoglobin, TEC, platelet count, and HCT values that were nearly comparable to the levels observed in the healthy control and sham-operated (SO) group. However, further investigations are needed to elucidate the reasons behind the alterations in platelet count in the diabeto-sepsis condition compared to diabetes and no changes in some hematological indices in diabeto-septic mice compared to sepsis.

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References

1. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, *et al*. Global, regional, and national sepsis incidence and mortality, 1990-2017: Analysis for the Global Burden of Disease Study. Lancet. 2020;395(10219):200-211.
2. Guarino M, Perna B, Cesaro AE, Maritati M, Spampinato MD, Contini C, *et al*. Update on Sepsis and Septic Shock in Adult Patients: Management in the Emergency Department. Journal of clinical medicine. 2023;12(9):3188.
3. Watkins JB, Sanders RA. Diabetes mellitus-induced alterations of hepatobiliary function. Pharmacological reviews. 1995;47(1):1-23.
4. Costantini E, Carlin M, Porta M, Brizzi MF. Type 2 diabetes mellitus and sepsis: state of the art, certainties and missing evidence. Acta diabetologica. 2021;58(9):1139-1151.
5. Schuetz P, Castro P, Shapiro NI. Diabetes and sepsis: Preclinical findings and clinical relevance. Diabetes care. 2011;34(3):771-778.
6. Sun M, Tao Y, Chen WM, Wu SY, Zhang J. Optimal statin use for prevention of sepsis in type 2 diabetes mellitus. Diabetology & metabolic syndrome. 2023;15(1):75.
7. Goyette RE, Key NS, Ely EW. Hematologic changes in sepsis and their therapeutic implications. Seminars in

respiratory and critical care medicine. 2004;25(6):645-659.
8. Arkew M, Yemane T, Mengistu Y, Gemechu K, Tesfaye G. Hematological parameters of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia: A comparative cross-sectional study. PloS one. 2021;16(6):e0253286.
9. Furman BL. Streptozotocin-Induced Diabetic Models in Mice and Rats. Current protocols in pharmacology. 2015;70:5.47.1-5.47.20.
10. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock - A review of laboratory models and a proposal. The Journal of surgical research. 1980;29(2):189-201.
11. Mishra SK, Choudhury S. Experimental Protocol for Cecal Ligation and Puncture Model of Polymicrobial Sepsis and Assessment of Vascular Functions in Mice. Methods in molecular biology (Clifton, N.J.). 2018;1717:161-187.
12. Aird WC. The hematologic system as a marker of organ dysfunction in sepsis. Mayo Clinic proceedings. 2003;78(7):869-881.
13. Kannan K, Choudhury S, Ramasamy T, Kesavan M, Parida S, Singh TU, *et al*. Erythropoietin prevents sepsis-induced clinical severity and haematological changes in mice. The Pharma Innovation Journal. 2020;9:81-84.
14. Bateman RM, Jagger JE, Sharpe MD, Ellsworth ML, Mehta S, Ellis CG, *et al*. Erythrocyte deformability is a nitric oxide-mediated factor in decreased capillary density during sepsis. American journal of physiology. Heart and circulatory physiology. 2001;280(6):H2848-H2856.
15. Drewry AM, Samra N, Skrupky LP, Fuller BM, Compton SM, Hotchkiss RS, *et al*. Persistent lymphopenia after diagnosis of sepsis predicts mortality. Shock. 2014;42(5):383-391.
16. Sáenz JJ, Izura JJ, Manrique A, Sala F, Gaminde I. Early prognosis in severe sepsis via analyzing the monocyte immunophenotype. Intensive care medicine. 2001;27(6):970-977.
17. Chung H, Lee JH, Jo YH, Hwang JE, Kim J. Circulating Monocyte Counts and its Impact on Outcomes in Patients With Severe Sepsis Including Septic Shock. Shock. 2019;51(4):423-429.
18. Sterner G, Carlson J, Ekberg G. Raised platelet levels in diabetes mellitus complicated with nephropathy. Journal of internal medicine. 1998;244(6):437-441.
19. Akah PA, Alemji JA, Salawu OA, Okoye TC, Offiah NV. Effects of Vernonia amygdalina on biochemical and hematological parameters in diabetic rats. Asian Journal of Medical Sciences. 2009;1(3):108-113.
20. Mahmoud AM. Hematological alterations in diabetic rats - Role of adipocytokines and effect of citrus flavonoids. EXCLI journal. 2013;12:647-657.