Dijlah Journal of Agricultural Sciences
Journal homage: https://djas.uowasit.edu.iq/index.php/djas/en

Research article

Protective Effects of Gum Arabic and Olive Leaf Aqueous Extracts on Hematological Alterations in Alloxan-Induced Diabetic Male Rats

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ARTICLE INFO

Keywords:
Alloxan-induced diabetes
Gum Arabic
Olive leaf extract
Hematological parameters
Leukocyte profile
Immunomodulation

Article history:
Received: 20th Jan 2026
Accepted: 1st Mar 2026
Online: 1st Apr 2026

ABSTRACT

Diabetes mellitus is often linked with hematological disorders and immune dysfunctions, which are mainly attributed to oxidative stress and inflammatory processes. The protective effects of gum Arabic and olive leaf aqueous extracts alone and in combination against hematological parameters in alloxan-induced diabetic male rats was evaluated. Forty-two adult male albino rats were randomly assigned into seven groups, six rats in each group (healthy control, diabetic control, diabetic rats treated with gum Arabic, diabetic rats treated with olive leaf extract, diabetic rats treated with gum Arabic treated with olive leaf extract, healthy rats treated with gum Arabic and healthy rats treated with olive leaf extract). A single i.p. dose of alloxan monohydrate (100 mg/kg body weight) induced diabetes. The extracts were orally administered for 4 weeks. At the end of the experimental period, hemoglobin concentration, packed cell volume, total white blood cell counts and differential leukocyte percentages were determined. The diabetic rats treated with alloxan exhibited significant haematological changes such as decrease in percentage lymphocytes and decrease in hemoglobin concentration, while the percentage neutrophils and percentage monocytes increased. These changes associated with diabetes were significantly improved with treatment using gum Arabic and olive leaf extract. Combined treatment exhibited the highest corrective effect as hemoglobin level was brought back to 15.57 ± 0.16 g/dL, PCV was restored to 38.43 ± 0.60% and TWBCC was returned to 7.93 ± 0.10 $\times 10^3/mm^3$. It also was beneficial in maintaining a balance in leukocytes, showing higher percentage of lymphocytes and a lower percentage of neutrophils and monocytes than the untreated diabetic rats. Neither extract caused any undesirable haematological effects in healthy rats. The results showed that both the gum Arabic and the olive leaf aqueous extracts alone or in combination could act as protective agents against the diabetic changes induced in the blood of rats by alloxan. Additional studies that include oxidative stress markers, histopathological evaluation, dose–response studies, and use of standard antidiabetic controls are suggested to further elucidate the mechanisms and therapeutic implications.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that involves a continuous hyperglycemia caused by inadequate insulin production, or by insulin not being used effectively, or by a combination

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of both. Long-lasting hyperglycemia has been linked to oxidative stress, inflammatory activation and the progressive dysfunction of various organs and physiological systems. Chemically induced diabetes is commonly used in experimental models in order to study the complications of diabetes and potential protective agents. One of the commonly used models is alloxan-induced diabetes, which is caused by the selective destruction of pancreatic β-cells primarily through the production of reactive oxygen species (ROS) that result in insulin deficiency and chronic hyperglycemia (Katsumata & Katsumatay, 1992; Alarcon – Aguilar et al., 2002). Diabetes can have significant effects on hematological homeostasis as well as metabolic effects. Changes in hemoglobin value, packed cell volume, total white blood cell count and differential white blood cell count have been noted in diabetic state. These shifts could be due to diminished erythropoiesis, decreased erythrocyte survival, oxidative damage to the erythrocyte membrane, inflammation, and immune dysfunction ((Al-Tamimi, 2014; Colak et al., 2012; ALRekabi et al., 2018). Systemic inflammation and oxidative stress are features of diabetes that are often associated with leukocytosis, neutrophilia, monocytosis and decreased proportions of lymphocytes Vozarova et al., 2001; Al-Moussawi. 2014; Al-Naili, 2013; Al-Ani. 2016; Rossetti et al., 1993; Al-Husseini et al., 2003). Thus, hematological parameters can be valuable to assess the systemic effects of diabetes and potential protective effect of natural therapeutic agents. Gum Arabic or acacia gum is an exudate from the Acacia species which is soluble in water. Mainly made up of complex polysaccharides and glycoproteins and known as a soluble dietary fiber (Verspohl, 2002; Ibrahim et al., 2013). The physicochemical and biological properties have made gum Arabic an even more interesting natural functional product. Gum Arabic has been reported to have beneficial effects in diabetes and associated complications through its role in delaying carbohydrate absorption, altering glucose intestinal handling, improving metabolic balance, antioxidant activity and anti-inflammatory activity Babiker et al., 2018; Nasir et al., 2016; Elnour 2018; Baien et al., 2020). These properties suggest gum Arabic can be used to support blood and immune parameters against diabetes induced oxidative and inflammatory disturbances. The leaves of olive trees (Olea europaea L.) are also loaded with biologically active molecules, including phenolic compounds like oleuropein, hydroxytyrosol, flavonoids and other antioxidant molecules (Ghanema & Sadek, 2012; de Bock et al., 2013; Hooijmans et al., 2014).

Olive leaf extract has been extensively studied for its antidiabetic, antioxidant, anti-inflammatory and immunomodulatory effects (Boaz et al., 2011; Rashidi et al., 2013; Ghanema & Sadek, 2012; Eltayef, 2017). The antioxidant activity of olive leaf extract has been reported to have beneficial effects on blood components against diabetic damage, several studies have reported the improvement of glucose metabolism and reduction of oxidative stress by olive leaf extract (Ghanema & Sadek, 2012; Zakri, 2015). The effects demonstrated suggest that olive leaf extract is a natural compound with potential to improve hematological and inflammatory diabetes-related complications. Gum Arabic and olive leaf extract were both investigated for their potential biological activities but little information is available about the combined effect on the hematological and leukocytic alterations under diabetic conditions. Diabetes induced hematological alterations are well correlated to oxidative stress and inflammatory state; therefore, the synergistic effect of soluble dietary fiber (gum Arabic) and plant-based phenolic extract (olive leaf) could be beneficial in protecting the hematological alterations. But this assumption has to be tested experimentally, with proper diabetic and non-diabetic control groups .

In view of this, the present study was designed to assess the effects of the aqueous extracts of gum Arabic and olive leaves (both individually and mixed together) on some haematological parameters in normal and alloxan diabetic male rats. A study was conducted to investigate the effect of these

extracts for improving erythrocytic and leukocytic changes associated with diabetes by measuring hemoglobin concentration, Packed Cell Volume, Total leucocyte count and differential leukocyte count. The use of healthy treated groups also enabled the preliminary estimation of the hematological safety of the both extracts under the experimental conditions.

2. Materials and Methods

2.1 Experimental animals

Forty-two healthy adult male albino rats (200-250 g) were obtained from the Central Animal House, College of Life Sciences, University of Kufa, Iraq. Animals were acclimatized for 2 weeks before the start of the experiment with standard laboratory rearing conditions: 20–25°C, 12 h light/12 h dark photoperiod, free access to standard laboratory chow and drinking water. The rats were acclimatized and then divided into seven experimental groups of six rats each. Institutional animal care guidelines were followed for all animal procedures.

2.2 Preparation of plant extracts

2.2.1. Preparation of gum Arabic aqueous extract

The Gum Arabic powder was collected from a commercial source and confirmed by authentication prior to use. The material was dissolved in distilled water and removed of visible impurities. Gum Arabic powder was dissolved in distilled water to prepare a 10% aqueous gum Arabic solution.Gum Arabic powder was dissolved in distilled water to form 10 g of aqueous gum Arabic solution. The prepared solution was used as the working extract for oral administration.

2.2.2. Preparation of olive leaf aqueous extract

Fresh olive leaves were taken from the olive trees in Wasit Governorate, Iraq. The actual collection date should be placed in correctly prior to submission. Leaves were washed with distilled water, shaken off and air dried for 3 days in shade at room temperature. The dried leaves were powdered in an electric grinder. The olive leaf powder was extracted in an aqueous medium for 50g in 250 mL of distilled water, so as to obtain a 20% stock aqueous extract. For oral administration the extract was filtered and diluted in distilled water to give the working concentration of 10%.

2.3. Induction of experimental diabetes

The rats in diabetic groups were subjected to overnight fasting for about 12 h to induce experimental diabetes. Diabetes was induced by a single dose of freshly prepared alloxan monohydrate (100 mg/kg body weight) into the intraperitoneal space. After the administration of alloxan, glucose solution (5%) was given to rats in drinking water for 24 h to minimize the possibility of acute hypoglycemic shock. Glucose test strip was used to measure the Fasting blood glucose level. Fasting blood glucose levels ≥200 mg/dL, together with other typical clinical diabetic symptoms, including polyuria and lethargy, were indicative of diabetes and included in the experiment.

2.4. Experimental design and treatment allocation

Once diabetes was confirmed, animals were randomly assigned to 7 experimental groups of 6 rats. Four weeks of treatments were given orally daily. The dose volume administered was modified

to ensure uniformity during the experimental period if required to accommodate body weight. The experimental design is presented in Table 1 with the treatments also allocated.

Table 1. Experimental design and treatment allocation.

Group	Health status	Treatment	Dose/concentration	Route	Duration
G1	Healthy	Distilled water	1 mL	Oral	4 weeks
G2	Diabetic	Alloxan only	100 mg/kg	i.p.	—
G3	Diabetic	Gum Arabic	10%, 1 mL	Oral	4 weeks
G4	Diabetic	Olive leaf extract	10%, 1 mL	Oral	4 weeks
G5	Diabetic	Gum Arabic + Olive leaf extract	**10% Gum Arabic (1 mL) + 10% Olive leaf extract (1 mL); total volume 2 mL**	Oral	4 weeks
G6	Healthy	Gum Arabic	10%, 1 mL	Oral	4 weeks
G7	Healthy	Olive leaf extract	10%, 1 mL	Oral	4 weeks

i.p., intraperitoneal.

For the combination group, the administered preparation consisted of a combined oral formulation containing 10% gum Arabic and 10% olive leaf aqueous extract. The total administered volume was 1 mL per rat per day.

2.5. Blood collection

The animals were anaesthetised at the end of the four week treatment period with the appropriate anaesthetic procedure. Cardiac puncture blood samples were taken with sterile syringes. About 2 mL of blood was drawn from each rat and the blood samples were placed in EDTA-coated tubes for haematological analysis. These tubes were shaken lightly to avoid clotting and then immediately taken to the laboratory for analysis.

2.6. Hematological analysis

Hematological parameters such as hemoglobin concentration, packed cell volume and total white blood cell count were performed in automated hematology analyzer. The hemoglobin, packed cell volume and total white blood cell count were expressed in g/dL, percentages and $\times 10^3/mm^3$, respectively. Peripheral blood smears were obtained for differential leukocyte count. Thin blood smears were made on clean glass slides, air-dried and stained with Leishman's stain. The stained smears were viewed under oil immersion microscopy. The percentages of lymphocytes, neutrophils, monocytes, basophils and eosinophils were determined.

2.7. Statistical analysis

Python statistical packages were used to analyze data. Data are presented as mean $\pm$ SD. Normality of data distribution was tested with Shapiro–Wilk test and homogeneity of variance was tested with Levene's test. If the assumptions of normality and homogeneity of variance were met, differences in experimental groups were calculated by one-way analysis of variance (ANOVA) and compared by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. The difference was deemed as significant at $P < 0.05$. The various superscript letters in the same column in the results table are used to signify differences between groups.

3. Results and Discussion

3.1 Effects of gum Arabic and olive leaf extracts on erythrocytic indices

The results on the effects of gum Arabic and olive leaf aqueous extracts on Hb level and PCV of the experimental animals are presented in Table 2 and Figures 1A and 1B. Erythrocytic indices were

considerably affected by alloxan-induced diabetes. The lowest levels of hemoglobin and PCV were observed in the diabetic control group (G2) with means of 12.85 ± 0.26 g/dL and 30.73 ± 0.86%, respectively. The results obtained were considerably lower than those in the healthy control group (G1) with 15.32 ± 0.41 g/dL Hb and 40.08 ± 1.32% PCV. Gum Arabic and olive leaf extract were able to restore the erythrocytic parameters that were reduced by diabetes. Hb level was found to be 14.98 ± 0.21 g/dL in diabetic rats treated with gum Arabic (G3) whereas the PCV level was increased to 32.90 ± 0.96% in the same group.

Table 2. Effects of gum Arabic and olive leaf aqueous extracts on hematological parameters in normal and alloxan-induced diabetic male rats.

Group	Group_Label	Hb (g/dL)	PCV (%)	WBC ($\times 10^3/mm^3$)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Basophils (%)	Eosinophils (%)
G1	Healthy control	15.32 ± 0.41a	40.08 ± 1.32a	6.73 ± 0.26f	58.5 ± 1.05a	5.5 ± 0.55c	34.67 ± 0.52f	0.67 ± 0.52a	0.83 ± 0.41b
G2	Diabetic control	12.85 ± 0.26d	30.73 ± 0.86e	11.5 ± 0.28a	36.83 ± 1.47f	9.0 ± 0.0a	60.33 ± 1.63a	0.33 ± 0.52a	2.17 ± 0.41a
G3	Diabetic + GA	14.98 ± 0.21b	32.9 ± 0.96d	9.75 ± 0.19b	41.17 ± 0.75e	7.33 ± 0.52b	50.5 ± 0.84b	0.67 ± 0.52a	1.5 ± 0.55b
G4	Diabetic + OLE	14.37 ± 0.27c	36.67 ± 0.86c	8.35 ± 0.23c	48.0 ± 1.41d	7.0 ± 0.63b	41.17 ± 0.98c	0.67 ± 0.52a	2.67 ± 0.52a
G5	Diabetic + GA + OLE	15.57 ± 0.16a	38.43 ± 0.6b	7.93 ± 0.1d	50.83 ± 1.17c	6.33 ± 0.52c	39.5 ± 0.84d	0.67 ± 0.52a	2.5 ± 0.55a
G6	Healthy + GA	15.25 ± 0.19a	39.17 ± 0.53a	7.28 ± 0.12e	55.83 ± 0.75b	5.33 ± 0.52d	35.83 ± 0.41e	0.67 ± 0.52a	1.67 ± 0.52b
G7	Healthy + OLE	15.23 ± 0.12a	40.42 ± 0.61a	7.7 ± 0.18d	52.67 ± 0.82c	6.0 ± 0.63c	36.67 ± 0.52e	0.33 ± 0.52a	2.67 ± 0.52a

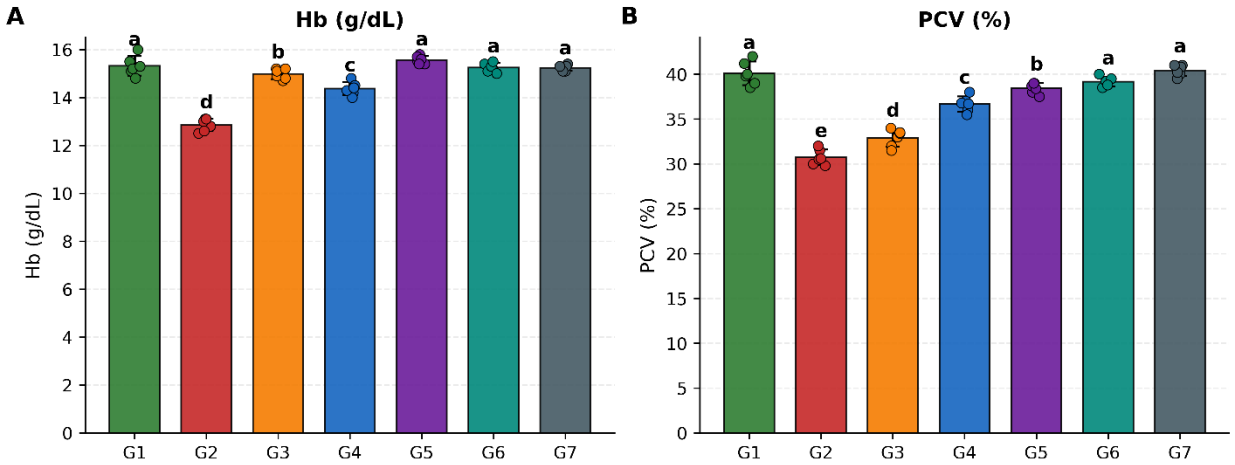

Figure 1. Effects of gum Arabic and olive leaf aqueous extracts on erythrocytic indices in normal and alloxan-induced diabetic male rats. (A) Hemoglobin concentration and (B) packed cell volume. Values are expressed as mean ± SD, n = 6. Different letters indicate significant differences among groups according to Tukey's HSD test at $P < 0.05$. G1: healthy control; G2: diabetic control; G3: diabetic + gum Arabic; G4: diabetic + olive leaf extract; G5: diabetic + gum Arabic + olive leaf extract; G6: healthy + gum Arabic; G7: healthy + olive leaf extract.

The same parameters were also improved in olive leaf extract treatment (G4) wherein the level of Hb was 14.37 ± 0.27 g/dL and the level of PCV was 36.67 ± 0.86%. Both the single treatments were

effective in improving the hematological status when compared to the diabetic control group, but the results were different for Hb and PCV. Olive leaf extract had a more significant effect on PCV and Gum Arabic had a more significant effect on Hb. Treatment with the combination of gum Arabic and olive leaf extract (G5) had the most dramatic recovery effect in the diabetic treated groups. The highest Hb value (15.57 ± 0.16 g/dL) was observed in this group and was statistically similar to the healthy control and healthy treated groups. There was also a significant improvement in the PCV values of the diabetic group as compared to the untreated diabetic group with the PCV value reaching 38.43 ± 0.60%. The results indicate that the synergic treatment was more effective than the individual treatments with either extract in reducing diabetes associated anemia-like changes. Both rats treated with gum Arabic (G6) and olive leaf extract (G7) were normal in their erythrocytic parameters. Hb values were 15.25 ± 0.19 g/dL in G6 and 15.23 ± 0.12 g/dL in G7, while PCV values were 39.17 ± 0.53% and 40.42 ± 0.61%, respectively. These values were similar to those of healthy controls, thereby showing that both extracts did not impair the erythrocytic indices in healthy animals.

3.2. Effects of gum Arabic and olive leaf extracts on total white blood cell count

The overall leukocytosis is displayed in Table 2 & Figure 2A. The Total WBC count was significantly increased in alloxan induced diabetic rats. The diabetic control group (G2) recorded the highest WBC count, 11.50 ± 0.28 $\times 10^3/mm^3$, compared with 6.73 ± 0.26 $\times 10^3/mm^3$ in the healthy control group.

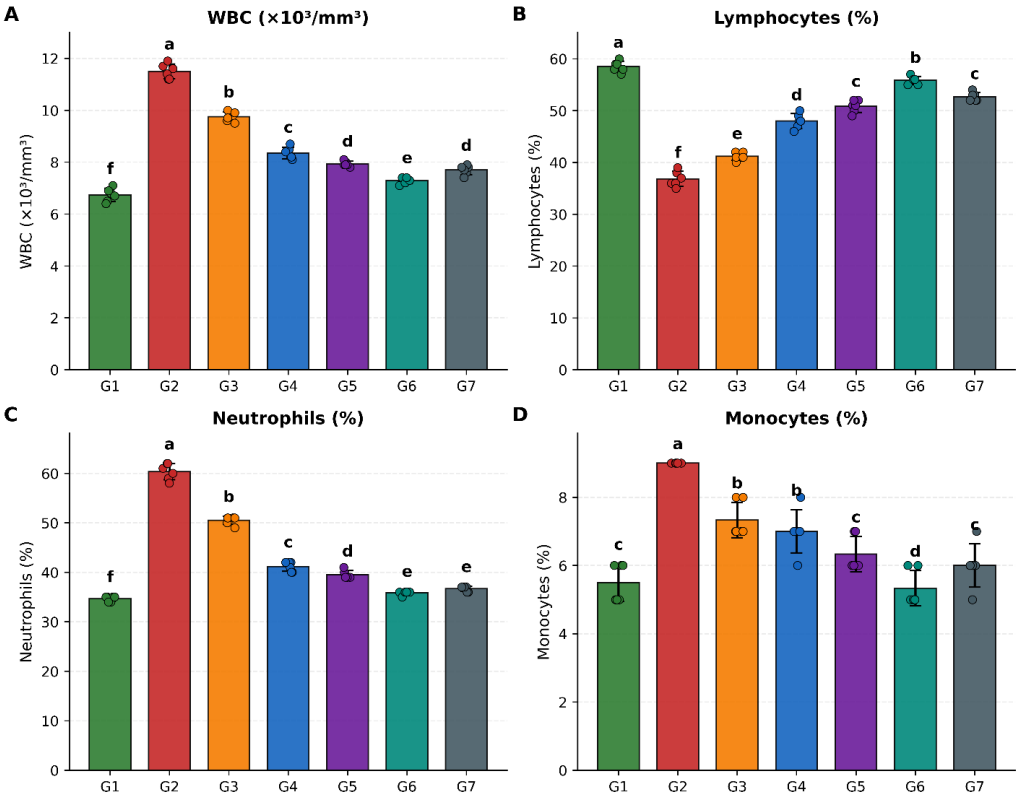

Figure 2. Effects of gum Arabic and olive leaf aqueous extracts on leukocyte profile in normal and alloxan-induced diabetic male rats. (A) Total white blood cell count, (B) lymphocytes, (C) neutrophils, and (D)

monocytes. Values are expressed as mean ± SD, n = 6. Different letters indicate significant differences among groups according to Tukey's HSD test at $P < 0.05$. G1: healthy control; G2: diabetic control; G3: diabetic + gum Arabic; G4: diabetic + olive leaf extract; G5: diabetic + gum Arabic + olive leaf extract; G6: healthy + gum Arabic; G7: healthy + olive leaf extract.

This increase suggests that there is a definite systemic inflammatory or immune-stress reaction in alloxan induced diabetes. In diabetic rats, the count of WBC was significantly reduced to $9.75 \pm 0.19 \times 10^3/mm^3$ with treatment of gum Arabic and $8.35 \pm 0.23 \times 10^3/mm^3$ with olive leaf extract. The simultaneous treatment additionally resulted in the lower level of WBCs ($7.93 \pm 0.10 \times 10^3/mm^3$) close to those of healthy groups. All these findings suggest a diabetes-associated leukocytosis was mitigated by both extracts where diabetic combined treatment had the strongest correcting effect among diabetic groups. In healthy treated groups WBC did not significantly deviate from normal physiological range. G6 recorded $7.28 \pm 0.12 \times 10^3/mm^3$, while G7 recorded $7.70 \pm 0.18 \times 10^3/mm^3$. The non-adverse leukocytic stimulation reported here is consistent with healthy animals being treated with either extract.

3.3. Effects on lymphocyte percentage

Table 2 and Figure 2B showed that the distribution of lymphocytes was significantly different between the experimental groups. The percentage of lymphocytes was the highest in healthy controls (G1) 58.50 ± 1.05%. The diabetic control group (G2), however, exhibited a significant decrease to 36.83 ± 1.47%, suggesting that diabetes induction significantly affected immune cell balance. The use of gum Arabic treatment resulted in improvement of the percentage of lymphocytes to 41.17 ± 0.75%, and olive leaf extract treatment resulted in a greater increase (48.00 ± 1.41%). The simultaneous treatment gave a better recovery of lymphocytes per cent (50.83 ± 1.17%) than the single treatments. The number of lymphocytes, however, did not normalize completely in G5 as compared to the healthy controls. In healthy rats, the treatment with gum Arabic kept the percentage of lymphocytes at 55.83 ± 0.75% whereas the treatment with olive leaf extract kept the percentage of lymphocytes at 52.67 ± 0.82%. The values were not as high as in the healthy control group, but were within a relatively high lymphocytic profile and were therefore not suggestive of adverse immune suppression.

3.4. Effects on neutrophil percentage

The value of neutrophils percentage was inversely proportional to lymphocytes, as shown in Table 2 and in Figure 2C. The diabetic control group (G2) had the highest neutrophil percentage of 60.33 ± 1.63% followed by the healthy control group with 34.67 ± 0.52%. This marked rise indicates that there is a strong neutrophil dominated inflammatory response, encouraged by the alloxan induced diabetes. Gum Arabic treatment caused a decrease in neutrophils to 50.50 ± 0.84%, whereas olive leaf extract caused a more pronounced decrease into 41.17 ± 0.98%. The treatment with the combination of both further reduced the percentage of neutrophils to 39.50 ± 0.84%, near to the healthy groups level. This means that the mixture of gum Arabic and olive leaf extract was very effective in normalizing the increased number of neutrophils in diabetes. The treated healthy groups had percentages of neutrophils that remained similar to the healthy control group. G6 and G7 recorded 35.83 ± 0.41% and 36.67 ± 0.52%, respectively. Both extracts were found to not cause a damaging neutrophilic response in healthy animals, confirming these results.

3.5. Effects on monocyte percentage

Diabetes induction also significantly altered the monocyte % as indicated in Table 2 and Figure 2D. In the diabetic group (G2), the highest percentage found was in the monocytes (9.00 ± 0.00%) compared to the healthy control group (5.50 ± 0.55%). This rise is consistent with the existence of an inflammatory response associated with diabetes. The percentage of monocytes was reduced to 7.33 ± 0.52% in the gum Arabic treated group and 7.00 ± 0.63% in the olive leaf extract treated group. The joint treatment led to an additional decrease to 6.33 ± 0.52%, revealing an enhanced effect than either treatment alone. The healthy rats treated with gum Arabic exhibited the lowest percentage of monocytes (5.33 ± 0.52%), and the healthy rats treated with olive leaf extract was 6.00 ± 0.63% of monocytes. The findings indicate that the extracts, mainly in combination, helped to regulate the inflammatory response of the monocytes that were triggered by alloxan diabetes.

3.6. Effects on basophils and eosinophils

The minor leukocyte fractions are shown in Table 2. There were no significant differences in the percentages of basophils in the groups. There were no significant differences between the groups in terms of the percentage of basophils. There was no definite change in the values of basophils in alloxan induced diabetes or administration of extract as shown in Figure 3A. There was only a small range of variation in eosinophil percentage between groups. Diabetic control rats had 2.17 ± 0.41% and healthy control group (G1) had 0.83 ± 0.41%. In G4 and G7 percentages of eosinophils were higher, 2.67 ± 0.52% and 2.50 ± 0.55% respectively, as shown in Figure 3B. There was some statistical difference, but the changes were small in absolute terms and should be viewed with caution, as the absolute eosinophil counts are small. Hence, eosinophils and basophils should be considered as secondary findings not primary endpoints.

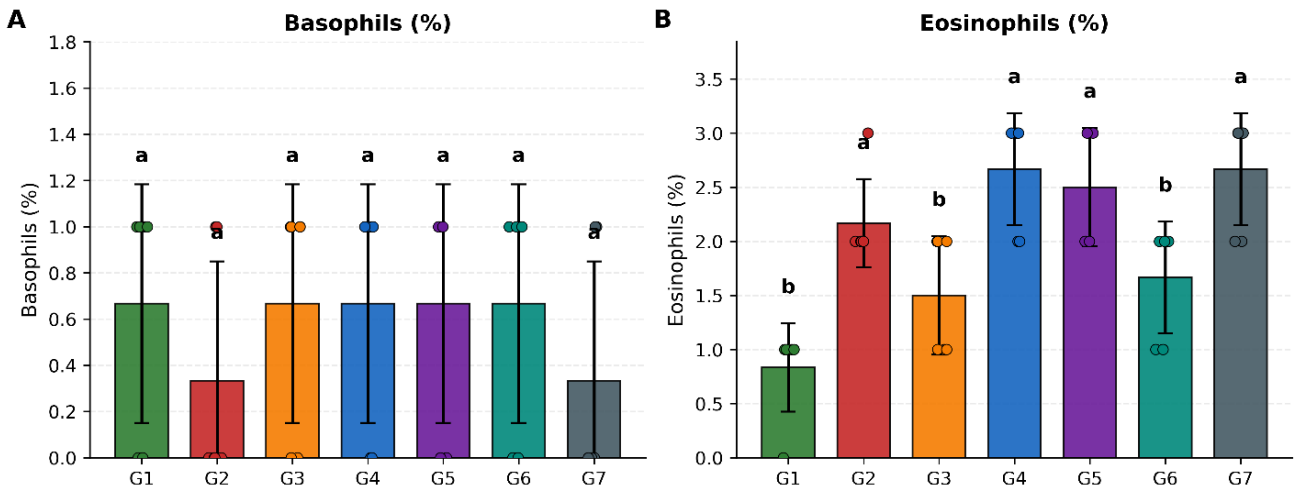

Figure 3. Effects of gum Arabic and olive leaf aqueous extracts on minor leukocyte fractions in normal and alloxan-induced diabetic male rats. (A) Basophils and (B) eosinophils. Values are expressed as mean ± SD, n = 6. G1: healthy control; G2: diabetic control; G3: diabetic + gum Arabic; G4: diabetic + olive leaf extract; G5: diabetic + gum Arabic + olive leaf extract; G6: healthy + gum Arabic; G7: healthy + olive leaf extract.

3.7. Overall hematological pattern

A heatmap is used to summarise the overall hematological pattern. The diabetic control group (G2) had a very distinct pathological profile where it showed a decrease in Hb, PCV and lymphocytes percentage and an increase in both WBC and monocyte and neutrophils percentage. This pattern was clearly different for G2 compared to healthy controls and validated the disrupting influence of alloxan diabetes on hematological and immune homeostasis as presented in Figure 4. There was a range of restoration within treatment groups. The main effects of gum Arabic treatment were an improvement in Hb levels and a partial decrease in inflammatory leukocyte changes. There was a greater effect of olive leaf extract on PCV, WBC count, percentage of lymphocytes and reduction in neutrophils. The level of recovery pattern in the combined treatment group was more balanced with improvement in erythrocytic indices and restoration of the leucocyte profile towards the pattern of the healthy control group. Healthy treated rats were very similar to the healthy controls, suggesting that neither gum Arabic nor olive leaf extract had significant deleterious hematological effects in non-diabetic rats in conditions tested in this experiment.

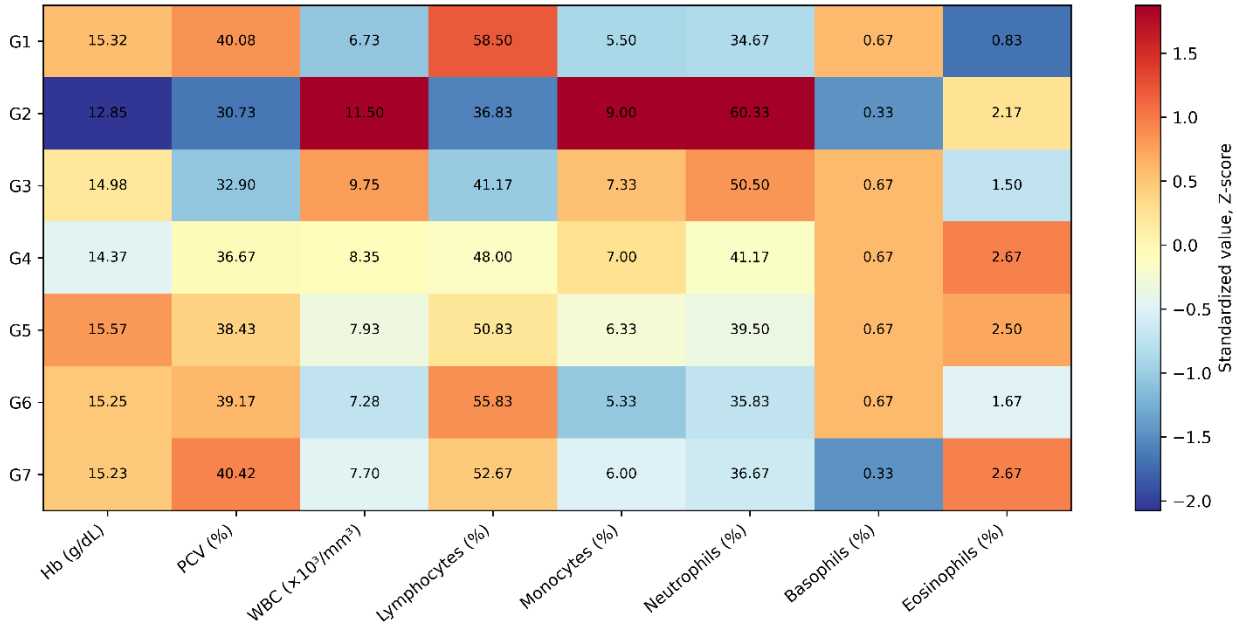

Figure 4. Heatmap of Hematological Responses Across Experimental Groups

The present study showed that the alloxan induced diabetes resulted in marked hematological changes, such as decreased value of hemoglobin (Hb), packed cell volume (PCV) and lymphocyte count, and increased total white blood cell (WBC), neutrophils and monocytes count. Both gum Arabic and olive leaf extracts were effective in significantly reducing these abnormalities when used individually or in combination, suggesting their potential protective and therapeutic effect against diabetes-induced hematological abnormalities.

The marked decrease in Hb and PCV noted in alloxan-treated diabetic group may reflect a deficient erythropoiesis and disturbance in hematological homeostasis. Alloxan has been described to inhibit DNA synthesis and subsequent protein synthesis and thus to inhibit erythrocyte maturation, causing a decrease in PCV levels (Rogers & Matossian-Rogers, 1982. In addition, the rather high percentage of immature and microcytic erythrocytes may be responsible for the noted reduction in PCV (Rogers &

Matossian-Rogers, 1982). This is consistent with previous observations in experimental diabetic models (Al-Tamimi, 2014). Lowered Hb concentrations can also be related to decreased iron mobilization from its main intracellular storage compartment, ferritin, caused by alloxan action on iron metabolism (Thomas & Aust, 1986).

Chronic hyperglycemia also causes oxidative stress that negatively impacts on survival of erythrocytes and iron homeostasis, causing a decrease in both hemoglobin concentration and PCV (Ganong, 1995). The results are parallel to those reported previously which showed hematological disturbances in diabetic animals (Colak et al., 2012; ALRekabi et al., 2018). Gum Arabic and olive leaf extract treatment significantly improved the level of Hb concentration compared to diabetic control with the highest effect recorded by the combination treatment. The reduction in the hematological indices after the administration of olive leaf can be explained by the elevation of its phenolic content, which has been found to stimulate the erythropoietic activity and improve the activity of enzymes involved in erythrocyte maturation and hemoglobin synthesis (Zakri, 2015). Similarly, the gum Arabic is rich in bioactive compounds with strong antioxidant properties that can help to prevent oxidative damage to hematopoietic tissues and stimulate repair processes. Hb concentration and PCV were increased back to the control levels after administration of the gum Arabic and olive leaves suggesting the synergistic effect of the two extracts. This has been noted elsewhere (Zakri, 2015); (Al-Ani, & Al-Kattan, 2020). In the present study it was also found that total WBC count also increased significantly in alloxan induced diabetic animals. Rabbits and rats treated with alloxan have been reported to have elevated leukocyte counts (Vozarova et al., 2001; Al-Moussawi, 2014; Al-Naili, 2013) in many instances, it is thought that these are a result of inflammatory and oxidative stress responses related to diabetes (Al-Ani, 2016).
The higher levels of total WBC may be due to higher neutrophils. Leukocyte migration and immune regulation have been reported to be impaired in diabetes and lead to increased circulation of neutrophils (Al-Tamimi, 2014; Rossetti et al., 1993). Furthermore, hyperglycemia may also induce inflammatory signaling to cause bone marrow activation and stimulate granulopoiesis, leading to production of neutrophils and other leukocyte populations (Al-Husseini et al., 2003). Diabetic group had significantly higher counts of total WBCs than those in the group treated with gum Arabic and olive leaf extracts. The antioxidant and anti-inflammatory properties of both extracts, such as phenolic compounds, flavonoids, and other bioactive phytochemicals (James et al., 2007) may explain this effect. Moreover, these extracts can help repair tissues where leukocytes are produced like the liver, spleen, and bone marrow which can help normalize leukocytes production (Sakr & Gabr, 1992).
Interestingly, the results showed that the treatment using the combined extract resulted in significantly lower WBC count than either extract alone, bringing the levels back to near the control group. This discovery further reinforces the idea of a synergistic effect of gum Arabic and olive leaves when used together. There was no significant leukocytic changes noted in the groups treated with the extracts alone, indicating that these treatments are relatively safe, and have no detrimental effect on hematological function. A slight increase in the number of WBCs in some treated groups might be associated with the immunostimulatory effect of phytochemicals like flavonoids and glycosides (Eltayef, 2017) as reported earlier (Baien et al., 2020). Differential leucocyte count showed there were marked increases in neutrophils and monocytes in diabetic animals. The changes are probably due to the activation of the innate immune system as a result of tissue injury caused by alloxan. Neutrophils are the first line of tissue damage and inflammatory defense; thus, an increased production of neutrophils may be a compensatory response to tissue damage (Highleyman, 2003; Marchi, 2014; Yapo, 2011).

Likewise, the recruitment and proliferation of monocytes are typical events of inflammatory and tissue remodeling diseases. In contrast, the diabetic group had markedly decreased lymphocyte counts. The decrease could be due to the emigration of lymphocytes to tissues and inflammatory sites as well as due to direct cytopathic effects of the oxidative stress resulting from alloxan injection (Naclerio et al., 2017). This inverse relationship between increased neutrophils and decreased lymphocytes is further indication of the inflammatory environment of experimental diabetes. Leukocytes profiles were significantly improved by gum Arabic and olive leaf extracts. Numerous changes towards immune homeostasis were observed, with a reduction in neutrophil and monocyte numbers, and an increase in lymphocytes, compared to the diabetic group. Such effects may be attributed to the antioxidant's components including tannins, saponins, phenols that can help to reduce oxidative stress, improve phagocytic activity, and repair tissues (Francis et al., 2002; Schulz et al., 2001). The anti-oxidant activity of these phytochemicals may be responsible for reducing the inflammatory cascade induced by alloxan and thus restore the leukocytes dynamics.

Overall, treatment with gum Arabic and olive leaf extracts was found to be the most effective treatment among all treatment groups. The combined treatment group had significant normalization of neutrophils and monocytes with values very close to the control group. The number of lymphocytes was not significantly reduced when compared to control levels but were significantly improved when compared to the diabetic group. The remaining lymphopenia could be due to the continued migration of lymphocytes to the sites of tissue damage and the continued repair process following alloxan damage. The results of the present study are corroborated by the previous studies that have shown that antioxidants present in plants have immunomodulatory and protective effect in diabetic models (Abbas, 2008). The present results indicated that both the gum Arabic and the olive leaf extracts had protective effects against the hematological disturbances caused by diabetes by acting as antioxidants, anti-inflammatory agents and tissue repair substances. Furthermore, the administration of both extracts seems to be more effective than either alone, implying a possible synergistic effect that needs to be studied.

4. Conclusion

Uncontrolled diabetes in rats caused by alloxan results in profound anemia and inflammatory stress throughout the body, which affect hematological and immune homeostasis. However, daily oral administration of 10% aqueous extracts of gum Arabic and olive leaf shows a significant therapeutic potential in reducing these diabetic complications. Single use of each extract produces a better effect on hemoglobin, packed cell volume and leukocytes; however, a synergistic effect is obtained when they are used together. This combination therapy normalizes red blood cell indices and restores the normal physiological balance between lymphocytes and neutrophils, and returns the immune parameters to a level that is statistically comparable to healthy and non-diabetic controls. Besides, the extracts given to healthy rats demonstrate their safety and full absence of hematological toxicity. The results highlight the strong anti-anemic and immunomodulatory effects of both natural extracts from plants. For this reason, their combined use as an adjuvant therapy is a highly attractive, safe and natural therapeutic approach for treating diabetes mellitus related hematological and immunologic impairments, which must be further investigated pharmacologically and clinically in human beings to confirm the therapeutic effectiveness of the combined application.

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