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# EFFECT OF *HELLENIA SPECIOSA* (J. KOENIG) S. R. DUTTA ON HEMATOLOGICAL INDICES OF ALLOXAN INDUCED DIABETIC RATS

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**Keywords:**

*Hellenia speciosa*, alloxan, Hematocrite, Hemoglobin

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**ABSTRACT:** The present study aimed to evaluate the effect of crude leaf extract of *Hellenia speciosa* on hematological parameters of alloxan-induced diabetic rats. The study was carried out using alloxan model of diabetes for a period of 28 days and at the end of the treatment rats were sacrificed and blood samples were collected for the estimation of hematological parameters. The results showed that the white blood cell (WBC) count was significantly ($p<0.05$) increased while red blood cell (RBC) and hemoglobin (Hb) were significantly ($p<0.05$) decreased in diabetic rats compared to normal rats. Treatment with the crude leaf extract of *Hellenia speciosa* ameliorated the imbalances in hematological parameters caused by alloxan. Moreover, Induction of diabetes does not show any significant change in the differential count, MCV, MCH, MCHC, MPV, HCT and Platelets does not show alterations in normal and diabetic control. Therefore, the crude leaf extract of *Hellenia speciosa* can be considered relevant in the management of the disease.

**INTRODUCTION:** Diabetes mellitus is a metabolic, endocrine disorder with different aetiologies, characterized by a malfunction in carbohydrate, protein, and fat metabolism resulting in complete or relative insufficiency of insulin secretion and action or both [1, 2]. The chronic increase in blood glucose level results in microvascular and macrovascular complications, which are associated with diabetes mellitus [3]. Diabetes mellitus is a multifactorial illness associated with abnormalities in lipoprotein metabolism [4], high basal metabolic rate, and high oxidative stress-induced damage [5].

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Diabetes is a chronic disorder that also causes alteration in Hematological parameters. Hematological changes associated with diabetes consist mainly of abnormalities in the function, morphology, and metabolism of erythrocytes, leukocytes, and platelets [6]. The underlying cause of the changes in the hematology in diabetes mellitus is mainly due to oxidative damage [7, 8].

Medicinal plants are used for curing various diseases because of their properties, such as low cost and fewer side effects [9]. The demands for medicinal plants are increasing and play an important role in health care management [10, 11]. Plants are the basis for the development of modern drugs and medicinal plants have been used for many years in daily life to treat diseases all over the world. Plants are the basis for the development of modern drugs and medicinal plants have been used in many years in daily life to treat diseases all over

the world [12]. Herbal medicine involves the use of herbs and plant parts (roots, stems, leaves, barks, or even fruits) to promote and improve health [13]. Many plant secondary metabolites possess anti-oxidant potential and have shown an ameliorative effect on oxidative stress-induced damage in diabetes [14].

The plant *Hellenia speciosa* is a member of the family Costaceae. It is an ornamental, perennial, succulent rhizomatous herb growing up to 2.7 meters in moist, clayey soil under moderate shade place [15]. The plant reproduces vegetatively by stem cutting, rhizomes, or division of clumps. The leaves of the plant are oblong, thick, spirally arranged, flowers large white cone-like terminal spikes with bright red bracts [16]. The leaves and rhizomes of *Hellenia speciosa* have been reported to possess steroid-diosgenin, which is anti-diabetic in nature. The leaves also possess hypoglycemic properties and insulin potentiating action in addition to decreasing blood glucose [17]. This study aimed to determine the effects of crude extract of *Hellenia speciosa* leaves on the hematological indices of alloxan-induced diabetic rats.

## MATERIALS AND METHODS:

**Chemicals and Drugs Required:** The chemicals and drugs used in the study were plant extract, Alloxan, Glucose, carboxymethyl cellulose (CMC), metformin, isoflurane (anesthetic agent) was purchased from Sigma-Aldrich, St. Louis, MO, USA. Animal restrainer (*e.g.*, Broom restraint, Plas Labs), Micrometer, glucometer (Accu-Check, Roche, Germany).

**Experimental Animals:** Adult Sprague Dawley rats (100-140g) were purchased from Biogen, Bangalore, India was used for the study. All animals were housed 6/cage and kept in the animal house for one week for proper acclimatization under the controlled condition of illumination (12 h light / 12 h darkness), and the temperature is ranging 20-25 °C and fed on a standard pellet diet and water/ filtered water *ad libitum*. Ethical clearance for the handling of experimental animals was obtained from Institutional Animal Ethics Committee (IAEC) constituted for the purpose and care of laboratory animals, and taken as per the guidance of the Committee for Control and Supervision on Experiments on Animals (CPCSEA No.: 971/bc/06/CPCSEA)

**Plant Material:** *Hellenia speciosa* was collected from Thrissur district of Kerala, identified and confirmed by the botanist Dr. S. Ravikumar, PG and Research Department of Plant Biology and Biotechnology, Presidency College, Chennai. Voucher specimen no. PCMRDRM2017001.

**Preparation of Plant Extract:** Preparation of extracts was done according to the method described by [18]. About 1g of freshly dried powder of *Hellenia speciosa* and plant materials were extracted with 20 ml ethanol 75%, acetone, chloroform, aqueous, and petroleum ether (Merck, extra pure) for 1 minute using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman no. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evaporator at 40°C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extracts was approximately 100%. The solution was stored at 18°C until use.

**Preliminary Phytochemical Screening:** The extract was subjected to a preliminary phytochemical screening test for the presence of secondary metabolites according to the method described by [19-21].

**Induction of Experimental Diabetes Mellitus:** After one week of the acclimatization, rats were injected once with a low-dose of alloxan (80mg/kg) to induce partial insulin deficiency. The glucose value was noted using a glucometer before alloxan injection. This is called Basal value. After 48-96 hours of alloxan injection, the rat's fasting blood glucose values (glucometer) were noted using tail-flick method. The animals would display hyperglycemia and glucose intolerance. Animals with similar degrees of hyperglycemia (mostly above 95mg/dl) were considered.

**Experimental Design:** According to their glucose value, animals were randomized and divided to groups as follows

- Group 1 - Normal Group
- Group 2 - Vehicle control (Untreated)
- Group 3 - Diabetic control (Untreated)

- Group 4 - Diabetic group + Metformin (350 mg/kg, p.o),
- Group 5 - Diabetic group + *Hellenia speciosa* (300mg/kg).

**Animal Blood Collection:** Animals were kept fasting for 12 hours on the day before glucose estimation. Then the animal's tail was flicked, fasting blood parameters were noted on Day 0 and every week (week 1, 2, 3, and 4) of the entire observation period.

**Collection of Blood and Preparation of Serum Samples:** After four weeks of treatment with fractions, blood samples were obtained from all animals in each group through cardiac puncture and placed in labeled sample bottles with drops of Ethylenediaminetetraacetic acid (EDTA) for determination of hematological parameters.

**Determination of Hematological Parameters:** Determination of hematological parameters such as Hemoglobin (Hb), Hematocrit (PCV), Red cell count (RBC), White blood cell count (WBC), Mean corpuscular hemoglobin (MCH), Mean corpuscular volume (MCV), Mean platelet volume (MPV), Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Reticulocytes were determined by the use of automated hematological analyzer (ADVIA-120, Siemens, India).

**Statistical Analysis:** Statistical comparison was done using one-way ANOVA followed by Dunnett's post hoc comparison when more than two groups are involved. P valve less than 0.05 was considered significant.

**RESULTS AND DISCUSSION:** Hematological parameters are altered in metabolic disorders like diabetes mellitus. This was confirmed by the present study. The effect of the extract on the hematological parameters of diabetic rats is presented in **Table 1, 2, 3,** and **4,** respectively. In the present study, total WBC is significantly higher ($p<0.05$) in diabetic control compared with normal control. Administration of alloxan significantly ($p<0.05$) increased and decreased WBC and RBC count respectively. Induction of diabetes also leads to changes in the RBC and Hb level. The values of red blood cells and hemoglobin for the diabetic control and diabetic vehicle control were found to be lower than ($p<0.05$) the values of control groups.

**TABLE 1: EFFECT OF CRUDE LEAF EXTRACT OF *HELLENIA SPECIOSA* ON WBC, RBC, Hb, HCT IN CONTROL AND EXPERIMENTAL RATS**

| Groups | WBC ($10^3$/µl) | RBC ($10^6$/µl) | Hb (g/dl) | HCT (%) |
|---|---|---|---|---|
| Normal control | 8.6 ± 2.2 | 8.7 ± 0.5 | 15.5 ± 1.1 | 34.0 ± 0.2 |
| Diabetic control | 10.6 ± 1.2* | 6.8 ± 0.5* | 8.9 ± 0.6* | 32.0 ±0.2 $^{NS}$ |
| Diabetic Vehicle control | 8.0 ± 1.5 | 6.5 ± 0.4 | 8.6 ± 1.1 | 33.1 ±0.3 |
| Metformin (350 mg/kg) | 8.6 ± 1.3 | 8.3 ± 0.5 | 12.3 ± 0.4 | 34.1 ± 0.2 |
| *Hellenia speciosa* (300mg/kg) | 7.4 ± 2.1$^{\#}$ | 8.4 ± 0.4$^{\#}$ | 13.8 ± 0.2$^{\#}$ | 33.0 ±0.5 $^{NS}$ |

Values are expressed as Mean (n=6) ± Standard deviation *P<0.05 as compared to normal control and $^{\#}$P<0.05 compared to diabetic control. NS- No significance

**TABLE 2: EFFECT OF CRUDE LEAF EXTRACT OF *HELLENIA SPECIOSA* ON LYMPHOCYTES, MONOCYTES AND RETICULOCYTES IN CONTROL AND EXPERIMENTAL RATS**

| Groups | Lymphocytes (%) | Monocytes (%) | Reticulocytes (%) |
|---|---|---|---|
| Normal control | 89.1 ± 3.4 | 1.3 ± 0.5 | 2.0 ± 0.2 |
| Diabetic control | 88.3 ± 2.8 $^{NS}$ | 1.7 ± 0.4 $^{NS}$ | 2.0 ± 0.2 $^{NS}$ |
| Diabetic vehicle control | 90.0 ± 1.7 | 1.3 ± 0.2 | 2.1 ± 0.1 |
| Metformin (350 mg/kg) | 88.4 ± 0.1 | 1.5 ± 0.3 | 2.1 ± 0.2 |
| *Hellenia speciosa* (300 mg/kg) | 87.3 ± 31 $^{NS}$ | 1.3 ± 0.2 $^{NS}$ | 2.0 ± 0.5 $^{NS}$ |

Values are expressed as Mean (n=6) ± Standard deviation *P<0.05 as compared to normal control and #P<0.05 compared to diabetic control. NS-No Significance

In **Table 1** the primary reasons for assessing the RBC is to check anemia and to evaluate normal erythropoiesis. The hemoglobin level indicates the amount of intracellular iron, and the hematocrite represents the volume of RBC in 100 ml of blood. The mean cell hemoglobin level is a significant index for folic acid, or vitamin $B_{12}$ need [22, 23]. The results showed that the extract dose-dependently and significantly ($p<0.05$) decreased the WBC count and increased the RBC count of rats. There

was a slight decrease in hematocrit value in the diabetic rats. However, treatment with *Hellenia speciosa* reverted the values close to the normal group. In **Table 3,** induction of diabetes does not show any significant change in the differential count (lymphocytes, monocytes, reticulocytes, neutrophils, basophils, and eosinophils).

**TABLE 3: EFFECT OF CRUDE LEAF EXTRACT OF *HELLENIA SPECIOSA* ON NEUTROPHILS, BASOPHILS AND EOSINOPHILS IN CONTROL AND EXPERIMENTAL RATS**

| Groups | Neutrophils (%) | Basophils (%) | Eosonophils (%) |
|---|---|---|---|
| Normal control | 7.8 ± 2.1 | 0.2 ± 1.0 | 1.7 ± 1.2 |
| Diabetic contro | 8.3 ± 1.9 $^{NS}$ | 0.1 ± 0.1 $^{NS}$ | 2.0 ± 1.0 $^{NS}$ |
| Diabetic vehicle contro | 7.2 ± 1.0 | 0.2 ± 0.3 | 1.4 ± 0.6 |
| Metformin (350mg/kg) | 8.7 ± 0.8 | 0.2 ± 0.1 | 1.3 ± 0.4 |
| *Hellenia speciosa* (300mg/kg) | 8.18 ± 0.2 $^{NS}$ | 0.2 ± 0.1 $^{NS}$ | 1.3 ± 0.7 $^{NS}$ |

Values are expressed as Mean (n=6) ± Standard deviation *P<0.05 as compared to normal control and #P<0.05 compared to diabetic control. NS-No Significance.

**TABLE 4: EFFECT OF CRUDE LEAF EXTRACT OF *HELLENIA SPECIOSA* ON MCV, MCH, MCHC, MPV, AND PLT IN CONTROL AND EXPERIMENTAL RATS**

| Groups | MCV (fl) | MCH(pg) | MCHC (g/dl) | MPV (fl) | PLT ($10^3$/µl) |
|---|---|---|---|---|---|
| Normal control | 52.8 ± 1.6 | 19.6 ± 1.6 | 33.5 ± 1.1 | 6.3 ± 0.9 | 923.2 ± 117.1 |
| Diabetic control | 53.2 ± 1.3$^{NS}$ | 18.1±1.1$^{NS}$ | 33.6 ± 1.7$^{NS}$ | 6.7 ± 1.1$^{NS}$ | 955.7 ± 87.7$^{NS}$ |
| Diabetic vehicle control | 53.2 ± 1.2 | 18.6± 1.5 | 33.9± 2.0 | 6.6 ± 0.5 | 893.8 ± 151.7 |
| Metformin (350 mg/kg) | 53.7 ± 2.1 | 19.0 ± 0.9 | 33.7 ± 2.0 | 6.4 ± 0.5 | 897.8 ± 161.8 |
| *Hellenia speciosa* (300 mg/kg) | 53.2 ± 1.5$^{NS}$ | 19.4±1.2$^{NS}$ | 34.2 ± 1.5$^{NS}$ | 6.3 ± 0.3$^{NS}$ | 920.8 ± 87.3$^{NS}$ |

Values are expressed as Mean (n=6) ± Standard deviation *P<0.05 as compared to normal control and $^{\#}$P<0.05 compared to diabetic control. NS- No significance

Alloxan-induced rats exhibited only slight variations in the levels of MCV, MCH, MCHC, MPV, and PLT when compared with the control group in **Table 4**. Also, treatment with *Hellenia speciosa* and metformin does not exhibit any significant alterations in the levels of these parameters when compared with diabetic control.

## DISCUSSION:

**Hematological Parameters:** Hyperglycemia in individuals with diabetes is also associated with changes in hematological parameters as well as micro and macrovascular complications. Hematological indices can be used to assess the extent of destructive effects on blood constituents of animals due to diabetes mellitus. Anemia and increased erythrocyte fragility are some of the conditions been associated with diabetes [24-27].

In the current study, reduced RBC count, hemoglobin concentration, and mean corpuscular hemoglobin level were observed in rats in the diabetic group compared to the normal control (NC) group, indicating normocytic, hypochromic anemia. This is consistent with earlier reports that observed a significant decrease in this parameter in alloxan-induced diabetic rats [28-30]. The Hematological parameters such as WBC, RBC, Hb, and HCT are discussed in our current study. WBC count is significantly higher in the diabetic group compared to normal groups. An increase in WBC is a nonspecific marker of inflammation. The plant extract is effective in lowering the WBC count in diabetic rats [31]. **Table 1** shows that there was a significant increase in the WBC count of the diabetic rats. The increased immune cell counts may be the manifestations of the low-grade inflammatory reactions associated with the atherosclerotic complications of diabetes mellitus [32].

The results indicate there is a significant decrease in the RBC and Hemoglobin level in alloxan-induced diabetic rats. The main reason to study RBC is to check anemia and to analyze the process of normal erythropoiesis. Hemoglobin level indicates the amount of intracellular iron, while hematocrit indicates the volume of RBC present in the blood that helps to determine the degree of anemia or polycythemia [22]. The results indicate there is a significant decrease in the RBC and Hemoglobin level in alloxan-induced diabetic rats. Hemoglobin level indicates the amount of intracellular iron, while hematocrit indicates the volume of RBC present in the blood that helps to determine the degree of anemia or polycythemia.

Pawar *et al.,* in 2006 reported that the hemato-logical parameters were restored to almost normal after the administration of *Hellenia speciosa* [33].

The decreased levels of RBC and Hb in diabetic rats indicate the presence of anemia in diabetic conditions [34]. The major factor contributing to anemia is the inability of the kidney to synthesis RBC due to decreased hemoglobin. It is also reported that the presence of anemia in diabetes may be due to increased non-enzymatic glycosylation of RBC membrane protein which can be correlated with hyperglycemia [35]. Reduction in RBC may be due to infection in the blood system. This result was in agreement with Elavarasi *et al.,* (2021), who reported the same effect in streptozotocin-induced diabetic rats [36, 37]. The decreased level of total hemoglobin is mainly due to the increased formation of glycated hemoglobin. Reduction in RBC causes a significant decrease in Hematocrit values among the diabetic experimental groups [23].

Reactive oxygen species have also been implicated in red cell damage [38]. A decrease in hemoglobin in diabetic groups is because of the formation of glycosylated hemoglobin [39]. Similar reports have been reported by some other researchers in different plant extracts [40, 41].

**CONCLUSION:** From the results of the above study, phytochemical analysis of *Hellenia speciosa* (J. Koenig) S. R. Dutta confirmed the presence of bioactive compounds. The crude leaf extract of *Hellenia speciosa* has the potential to ameliorate the derangements in hematological indices associated with diabetes mellitus. The crude leaf extract of *Hellenia speciosa* exhibited beneficial effects on hematological parameters such as red blood cells, white blood cells, hemoglobin concentration of rats. Therefore, we conclude the plant extract can be used for the treatment of diabetes. However, the active principles in the extract and their mechanism of action should be delineated.

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