Study of Effect of *Allium cepa* extract on Haematological Parameter of Alloxan-treated Albino Mice (*Mus musculus*)

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Abstract

Allium cepa is considered as herbal medicine. To investigate the effect of *Allium cepa* extract on diabetic mice was considered. Total 24 Albino mice (*Mus musculus*) were divided into three groups: a control group (8 individuals) and two experimental groups (8 individuals in each group). Mice of the control group were fed a normal diet with distilled water, no addition of *Allium* extracts to their diet. Mice of the experimental group were treated with Allaxon. The first experimental group of mice received freshly prepared aqueous extracts of *Allium cepa* for 28 days and the second experimental group didn't receive Allium extract although they received a normal diet and distilled water like the control group. Red blood cell count, hemoglobin concentration, packed cell volume, and mean corpuscular volume, mean corpuscular hemoglobin concentration were determined after 28 days from administration of 1st dosage. Results showed that the Allium extract had a positive impact on the levels of the monitored hematological parameters. In the mice from the first experimental group, higher red blood cell count (4.77 million RBC/mm^3), hemoglobin concentration (11.9gm%), and corpuscular volume(35.7%) were found. In the second and experimental group red blood cell count(3.31 million RBC/mm^3), hemoglobin concentration(5.2gm%), and corpuscular volume(15.6%) were found lower than the control group.

Keywords: *Albino mice, Alloxan, Allium cepa, RBC, Hemoglobin*

Introduction

Allium cepa is frequently used as food supplements and provides a variety of health advantages because of its bioactive components. (Corzo-Martinez *et al*., 2007). Among the species in the genus Allium, onions (*Allium cepa*) have long enjoyed a distinct reputation as preventative and curative agents. Due to their rich vitamin, trace elements, amino acids, and organosulfur chemical content, onions (*Allium cepa*) and garlic (Allium sativum) have been used medicinally for over 5000 years. They play a well-known role in treating disorders of the cardiovascular. It is extensively used as an antibacterial drug, but it also has beneficial against cancer, diabetes, hypertension, platelets, and depression. It also has neuroprotective, anti-inflammatory, and antiparasitic properties (Chakraborty *et al*., 2022).

Chronic hyperglycaemia and anomalies in the metabolism of carbohydrates, lipids, and proteins as a result of deficiencies in insulin secretion, action, or both describe a set of metabolic illnesses known as diabetes mellitus (American Diabetes Association, 2014). Uncontrolled diabetes mellitus (DM) is linked to a number of illnesses, including cellular, metabolic, and blood parameters alteration that can cause vascular abnormalities (Agu, 2018). Diabetes is a metabolic syndrome, which also includes dyslipidaemia, obesity, hypertension, and alterations in haematological parameters (Karaman *et al*., 2009) Haematological changes encountered in DM patients include changes in the function, structure, and metabolism of RBCs (red blood cells), WBCs (white blood cells), PLT (platelet) and the coagulation systems(Antwi-Baffour *et al*., 2018).

Method & Material

Animals

Albino mice weighing 20–25gm were kept in polypropylene cages with a 12-hour light/12-hour dark cycle and a temperature range of 25–28^0 C. The rats had free access to food and tap water.

Preparation of Onion(*Allium cepa*) Extracts

Allium cepa bulb will be used for the study so, fresh onion bulb will be collected and cut into small pieces after that mashed it in stone grinder and squeezed it then, extract will be squeezed out (Anthony E. et. al 2015).

Study Design

Hyperglycemia will be induced by overnight fasted animal by a single intraperitoneal injection of alloxan (60 mg per kg body weight). 48 hour later hyperglycemia will be observed in model animal. Animal with equal or more than 160 mg/dl blood glucose in fasting state, will be selected for the experiment and consider it diabetic mice. (Jhon J. 2017).

To investigate the effect of *Allium cepa* extract on diabetic mice was considered. Total 24 Albino mice (*Mus musculus*) were divided into three groups: a control group (8 individuals) and two experimental groups (8 individuals in each group). Mice of the control group fed normal diet with distilled water, no addition of Allium extracts to their diet. Mice of the experimental group were treated with Alloxan. The first experimental group of mice received freshly prepared aqueous extracts of *Allium cepa* (0.6 gram/100 gram body weight) for 28 days and the second experimental group didn't receive Allium extract although they received a normal diet and distilled water like the control group.

Haematological Assay

Collection of Blood Sample: Blood samples were collected from the tail vein of *Mus musclus* after completion of dosage.

Estimation of Red Blood Cell Count: RBC Count were done by the routine method by the help of Neubauer slide.

Estimation of Hb: Estimation of Haemoglobin percentage was done by Sahli's method.

PCV: PCV were determined by Wintrobe's Haematocrit method.

MCV and MCHC were calculated by the following standard formula.

MCV(fl): PCV/RBC ×10

MCHC(%): Hb(100mg blood)/PCV ×100

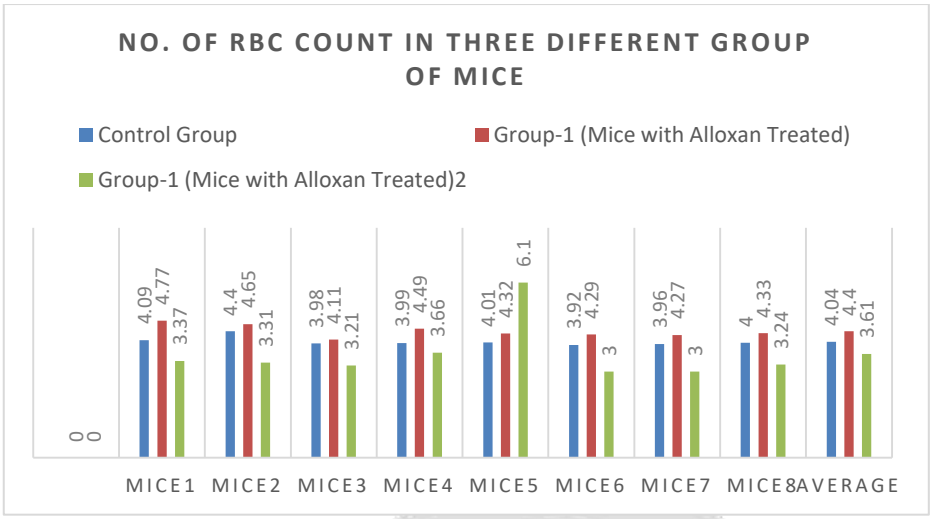
RESULT

The following results were obtained after the investigation

RBC Count

Sl. No.	Control Group	Group-1 (Mice with Alloxan Treated) Allium cepa Extract Provided to this group as Treatment	Group-1 (Mice with Alloxan Treated) No any kind of extract provided
Mice1	4.09	4.77	3.37
Mice2	4.40	4.65	3.31
Mice3	3.98	4.11	3.21
Mice4	3.99	4.49	3.66
Mice5	4.01	4.32	3.10
Mice6	3.92	4.29	3.00
Mice7	3.96	4.27	3.00
Mice8	4.00	4.33	3.24
Average	4.04 ± 0.053	4.40 ± 0.076	3.24 ± 0.36

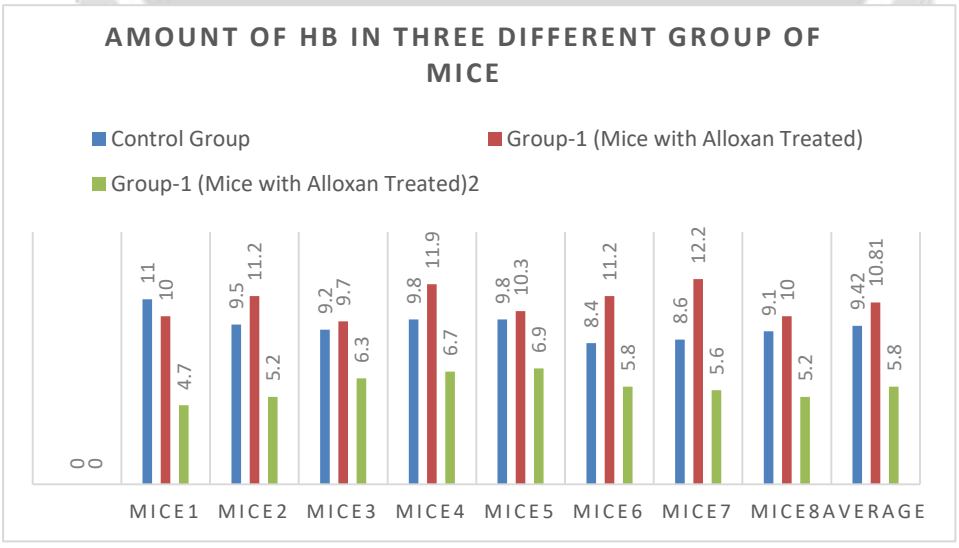
Table1: Total No of RBC Count in Different Groups in Mice (In Million/mm^3)



Hb Count

Sl. No.	Control Group	Group-1 (Mice with Alloxan Treated) Allium cepa Extract Provided to this group as Treatment	Group-1 (Mice with Alloxan Treated) No any kind of extract provided
Mice1	11	10	4.7
Mice2	9.5	11.2	5.2
Mice3	9.2	9.7	6.3
Mice4	9.8	11.9	6.7
Mice5	9.8	10.3	6.9
Mice6	8.4	11.2	5.8
Mice7	8.6	12.2	5.6
Mice8	9.1	10.0	5.2
Average	9.42± 0.28	10.81 ± 0.33	5.8 ± 0.27

Table2: Total Amount of Hb Count in Different Groups in Mice (In gm%).



PCV, MCV and MCHC

Group	PCV(%)	MCV (fl)	MCHC (%)
Control Group	29.8 ± 0.01	51± 0.08	30 ± 0.09
Group-1 (Mice with Alloxan Treated) Allium cepa Extract Provided to this	35.7 ± 0.12	55 ± 0.13	36 ± 0.16

group as Treatment			
Group-1 (Mice with Alloxan Treated) No any kind of extract provided	15.6 ± 0.14	38 ± 0.07	25 ± 0.07

Table3: Total Amount of PCV, MCV and MCHC in Different Groups in Mice

Parameters	**Test of Significance {Control group compared with Group-1 (Mice with Alloxan Treated) Allium cepa Extract Provided to this group as Treatment} p< 0.05**
RBC	The t-value is -3.83615. The p-value is .000908
Hb	The t-value is -3.1473. The p-value is .003565.
PCV	The t-value is -3.7458. The p-value is .000014
MCV	The t-value is -4.1142. The p-value is .000041
MCHC	The t-value is -3.2456. The p-value is .000024

Table4: Test of Significance Different haematological parameters {Control group compared with Group-1 (Mice with Alloxan Treated)Allium cepa Extract Provided to this group as Treatment} p< 0.05

DISCUSSION

Haematological alteration is a well-documented consequence of DM and is a significant and underappreciated burden in individuals (Christian et al. 2015). In the present study, there was a statistically significant difference in the total RBC count of diabetic mice compared to the control and experimental groups provided with *A.cepa* extract. The mean RBC count was lower (3.24 ± 0.36) in the diabetic mice group as compared to the control group (4.04 ± 0.053), and the difference was statistically significant (t value= 8.58731 and p value is .00001). In expt. group the mean value of RBC (4.40 ± 0.076) was increased significantly (t-value is -3.83615. The p-value is .000908) which indicates that the *A, cepa* extract may affect the no RBC in diabetic mice group. This increased ROS production and nonenzymatic glycosylation of Hgb and RBC membrane proteins, which results in reduced deformability, increased aggregation, and faster ageing of RBCs, could be the reason for the decreasing RBC count (Asmah et al., 2015, Cho *et al*. 2008 & Abdel & Hamed, 2016). It has also been demonstrated that these RBC alterations significantly raise blood viscosity, which negatively impacts microcirculation in diabetes and results in microangiopathy(Cho *et al*., 2008).

In the current study, we observed that the Hb gm percentage also decreases in diabetic mice(5.8± 0.27) in comparison to control mice (9.42± 0.28), but it increases(10.81 ± 0.33) significantly(t-value=-3.1473.p-value=.003565) in those diabetic mice that were provided *A cepa* juice. The current study showed a mild prevalence of anaemia among diabetic mice. In addition to renal disease, the aetiology of anaemia in diabetes is multifaceted and includes chronic hyperglycemia, inflammation, oxidative stress, AGEs, dietary deficiencies, medications, and hormonal changes (Sahay et al., 2017, Abdel & Hamed, 2016) Increased inflammatory cytokines including interleukin 6 (IL-6) and interleukin (IL-1) are indicative of chronic inflammation in diabetes. These pro-inflammatory cytokines may alter the sensitivity of erythroid progenitors to erythropoietin, encourage the death of immature RBCs, and reduce the number of circulating RBCs, leading to anaemia of inflammation (Sahay et al., 2017).

In table no 3 it can be clearly seen that the PCV, MCV and MCHC were significantly (p< 0.05) decreased in untreated diabetic mice comparison to control group mice, but it increased in A. cepa treated diabetic mice. Stookey *et al*., (2007) have shown that streptozotocin reduces the synthesis of MCH and MCHC, which indicates a defect in haemoglobin synthesis and a defect in osmotic pressure control and osmolality of the plasma.

It is clear that the RBC count, Hbgm% and other haematological parameters were improved in diabetic mice treated with *A.cepa*. First, chemicals found in *A. cepa* appear to have a stimulating action along certain pathways on a few hemopoietic growth factors' (cytokines') surfaces that interact with particular receptors to control the proliferation and the maturation and operation of adult cells, as well as the differentiation of progenitor cells. Normal For instance, erythropoiesis depends on the presence of sufficient erythropoietin levels together with other things. The end product of garlic and onion metabolism in the body may speed up Hb synthesis and RBC production by their indirect effect on erythropoietin. Chemical components of garlic and onion seem to act as active oxygen scavengers in vivo. It is therefore possible that they compete with haemoglobin in the RBC for oxygen resulting in tissue hypoxia which in turn stimulates the kidney directly to cause formation and secretion of erythropoietin (Samson et al.,2012).

Second, it's thought that the chemicals in *A. cepa* aid to enhance iron metabolism. Over 2 million RBC are produced by the bone marrow every second. Because iron is required for the synthesis of haemoglobin and is hence essential, this enormous effort can only be accomplished when there is sufficient iron available. The

bioavailability of iron from dietary grains, however, appears to be promoted by chemicals found in *A. cepa*, according to new research from India. They contend that these alliums can increase the body's ability to absorb iron from cereals and pulses. This is conceivable because the diallyl sufides in onions and garlic may trigger ferroportin mRNA, which can lead to an increase in the production of ferroportin, which is present on the plasma membrane of reticuloendothelial cells and the basolateral surface of gut enterocytes (Samson et al.,2012).

CONCLUSION

From the above study, we can conclude that Diabetes alters the haematological parameters. It reduces the no RBC, Hb% and other parameters in untreated diabetic mice but, when we treat diabetic mice with *A. cepa* extract the number of RBC and amount of Hb comes in not only normal conditions even better than the control mice.

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