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Impacts of aqueous extracts of *Annona senegalensis* and *Hallea ledermannii* on hematological parameters in Wistar rats made diabetic by alloxan induction.

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Abstract

Background: The costly treatment of diabetes mellitus is leading low-income populations to turn to phytotherapy, which requires preclinical testing to prevent dangerous side effects.

Objective: To evaluate the impact of aqueous extracts of *Annona senegalensis* (EAAs) and *Hallea ledermannii* (EAHl) on the haematological parameters of diabetic Wistar rats.

Material and methods: Diabetes was induced in normal rats by injection of alloxane. Diabetic rats were treated with the test substances for four (4) and thirteen (13) weeks. Haematological parameters were assayed after these treatments.

Results: Significantly, after four (4) weeks of treatment, platelet values increased by (49.54%) in animals treated with EAAs100. White blood cell count increased by 41.12% and 60.66% in rats treated with EAHl200.

After 13 weeks, in rats treated with EAHl400, an 84.32% increase ($p<0.05$) in platelets was observed. Neutrophil counts were statistically reduced by 34.92%, 53.97%, 49.21% and 53.97% respectively in diabetic rats treated with Gli10, EAAs100, EAAs200 and EAHl400, all compared with untreated diabetic rats.

Conclusion: This study showed that EAAs and EAHl have the capacity to fight infections in the body by helping to regulate haematological parameters.

Keywords: *Annona Senegalensis*; *Hallea ledermannii*; Glibenclamide; Hematology; Rats; Diabetes.

1. Introduction

Medicinal plants play a very important role in combating the majority of diseases in sub-Saharan Africa (Ladoh-Yemeda et al., 2016). They have therefore become essential for the treatment of many pathologies. Indeed, they contain phytochemical compounds that give them these properties (Haidara et al., 2020). For example, medicinal plants are used to treat metabolic diseases such as diabetes, hypertension, etc. (Halimi et al., 2016). Today, these diseases are a threat to human health. The management of these pathologies by modern medicine, is costly and often requires polytherapy (Houmènou et al.,2018). There is therefore a need to find new, less expensive treatments with less harmful effects than synthetic drugs. Hence the interest in plants frequently used in traditional medicine and renowned for their

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efficacy on many diseases (Embeya et al., 2019). According to the WHO (2010), 80% of the population uses medicinal plants to treat themselves.

These traditional therapeutics, with their little-known chemical compositions and pharmacological properties, are increasingly being offered to diabetics. It therefore seems essential for scientists to carry out ethnobotanical, phytochemical and pharmacological studies to validate the therapeutic virtues attributed to these preparations. With this in mind, we set out to study the effects of aqueous extracts of *Annona senegalensis (Annonaceae)* and *Hallea ledermannii (Rubiaceae),* two plants in the traditional African pharmacopoeia used in the treatment of diabetes (lawin et al., 2016; Njapdounke et al., 2016).

The roots and leaves of *Annona senegalensis* are used in decoction to treat diabetes (lawin et al., 2016). The fruit of this plant is used to treat kwashiorkor and marasmus (DALZIEL et al.,1937). It also treats headaches and body aches (ARNOLD et al., 1984; Chhabra et al.,1987). Authors such as (Diallo et al.,1995) have shown that *Hallea ledermannii* possesses anti-tumor activity. The plant also has antimicrobial and antioxidant properties (Adesegun et al., 1912).

The present study was initiated to investigate the impact of the effects of aqueous extracts of *Annona senegalensis (Annonaceae)* and *Hallea ledermannii (Rubiaceae)* on haematological parameters in diabetic rats.

The acute toxicity of aqueous extracts of (EAAs) and (EAHl) was investigated using the Organisation for Economic Co-operation and Development (OECD, 2001) guideline for the testing of chemicals.

2. Materials and methods

2.1. Plant material

The plant species used were *Annona senegalensis (Annonaceae)* and *Hallea ledermannii (Rubiaceae).* Fresh leaves of these plants were obtained from Bouaflé (central town, Côte d'Ivoire) and Yopougon (northern suburb of Abidjan, Côte d'Ivoire) respectively, and were identified and authenticated at the Centre National Floristique (CNF) of the Université Félix Houphouët-Boigny by Professor Aké-Assi.

Samples of *Annona senegalensis (Annonaceae)* and *Hallea ledermannii (Rubiaceae)* are preserved respectively under herbarium numbers 9809 Lamto 06/12/1967 and 2538 Forêt du banco 14/10/1954 at this center.

2.2. Preparation of aqueous extracts

Three hundred (300) grams of dried *Annona senegalensis* or *Hallea ledermannii* leaves, cut into pieces, are boiled for 1 hour in 1.5 liters of distilled water. The resulting decoctate, filtered several times through absorbent cotton, is oven-dried at 60°C. The aqueous extraction method produced yields of 18 g (7.2%) and 22.5 g (9%) for Annona senegalensis and Hallea ledermannii respectively. The powders obtained, stored in the refrigerator, constitute the aqueous extracts and were used in the experiments.

2.3. Animal material

The experiments were carried out on healthy male rats of the species Rattus norvegicus of the Wistar strain, with a body weight of between 200 and 250 g. The rats were reared at the UFR Biosciences animal house of the Université Félix Houphouët-Boigny at room temperature (25°C). The animals had access to water and food (pellets) ad libitum. Rats were acclimatized to a 12-hour day/night cycle prior to any experiment. Animals were treated in accordance with ethical rules concerning the use of laboratory animals.

3. Methods

3.1. Diabetes induction

Ninety-one (91) healthy male Wistar rats weighing between 200 and 250 g were used for diabetes induction. They were divided into seven (7) batches of thirteen (13) rats. After measuring their baseline blood glucose levels, which averaged between 72 ± 12 and 89 ± 11 mg/dl (i.e. healthy), they were given a single intraperitoneal dose of alloxane (75 mg/kg bw), diluted in 0.9% physiological sodium chloride solution. Seventy-two (72) hours later, blood glucose levels were measured after induction of diabetes. Rats presenting frank and permanent hyperglycemia between 158 and 238 mg/dl

were considered diabetic (Ndomou et *al*.,2014). Seventy (70) rats showed permanent hyperglycemia ranging from 173 ± 44.46 to 416.8 ± 82.19 mg/dl. These diabetic rats were used in our experiments.

3.2. Study of extract effects in diabetic rats

In this study, treatment with the aqueous plant extract, the reference product glibenclamide or distilled water began 24 hours after confirmation of diabetes. The test substances are administered daily over a period of 4 and 13 weeks. To assess the effects of the test substances, diabetic animals were divided up as follows:

- Batch 1, non-diabetic control rats: these rats received two (2) ml of distilled water daily by gavage;
- Batch 2, untreated diabetics: these rats received two (2) ml of distilled water daily by gavage;
- Lot 3, Diabetics + Gli10: these rats received daily by gavage two (2) ml of Glibenclamide solution dosed at 10-2 mg/kg bw;
- Lot 4, Diabetic + EAAs100: these rats received daily by gavage two (2) ml of the aqueous extract of Annona senegalensis dosed at 100 mg/kg bw;
- Lot 5, Diabetic + EAAs200: rats receiving daily oral administration of two (2) ml of Annona senegalensis aqueous extract at 200 mg/kg bw;
- Lot 6, Diabetic + EAHl200: these rats received daily by gavage two (2) ml of Hallea ledermannii dosed at 200 mg/kg bw;
- Batch 7, Diabetic + EAHl400: these rats received daily oral doses of 2 ml Hallea ledermannii at 400 mg/kg bw.

3.3. Blood sampling from diabetic rats

Blood is collected (about five (5) ml) from the tail vein of each rat by puncture, into tubes containing heparin, for haematological tests. Blood samples were taken from fasting rats (18 hours) before the start of the experiment (D0) (i.e. 72 hours after alloxane injection), then successively after (D56) and 13 (D91) weeks of treatment.

However, on day 91, blood sampling was carried out after some rats had been sacrificed by decapitation following ethyl urethane anaesthesia (Bouafou et al., 2007). Blood in anticoagulant-free tubes was centrifuged at 3,000 rpm for 10 min, in a refrigerated centrifuge (Alresa Orto, Spain) at 4°C. The serum is then collected and transferred to Eppendorf tubes for storage in a freezer (0°C), pending determination of hematological parameters.

3.4. Evaluation of hematological parameters in the blood of diabetic rats

The parameters studied are red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), hematocrit (Hte), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCHC), mean corpuscular hemoglobin concentration (MCHC), platelets, neutrophils, monocytes and lymphocytes.

The method used is the hemogram, also known as the complete blood count (CBC), which is carried out on a Mindray 5380 automated system (Model BC 5380RS6A Bis 1435, France).

3.5. Statistical analysis

Statistical analysis of values and graphical representation of data were performed using Graph Pad Prism 5 software (San Diego, California, USA).

Statistical differences between results were determined by analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test, with a significance level of $p < 0.05$.

4. Results

4.1. Effects of extracts after four (4) weeks of treatment

Red blood cell, haemoglobin and haematocrit levels and erythrocyte indices in diabetic rats (treated or untreated) did not vary significantly ($p > 0.05$) compared with values in non-diabetic control rats (Figs. 1 and 2).

After four (4) weeks of treatment, blood platelet levels decreased by 38.27 and 36.29%, respectively, in untreated diabetic rats ($p < 0.01$) and in those treated with EAAs200 ($p < 0.05$), compared with non-diabetic controls. The value of the same parameter increased significantly (49.54%) in EAAs100-treated animals compared with untreated diabetic rats (Figure 3).

The white blood cell count was very significantly increased by 41.12% and 60.66% in EAHl 200-treated rats compared with non-diabetic and untreated diabetic rats respectively (Figure 4).

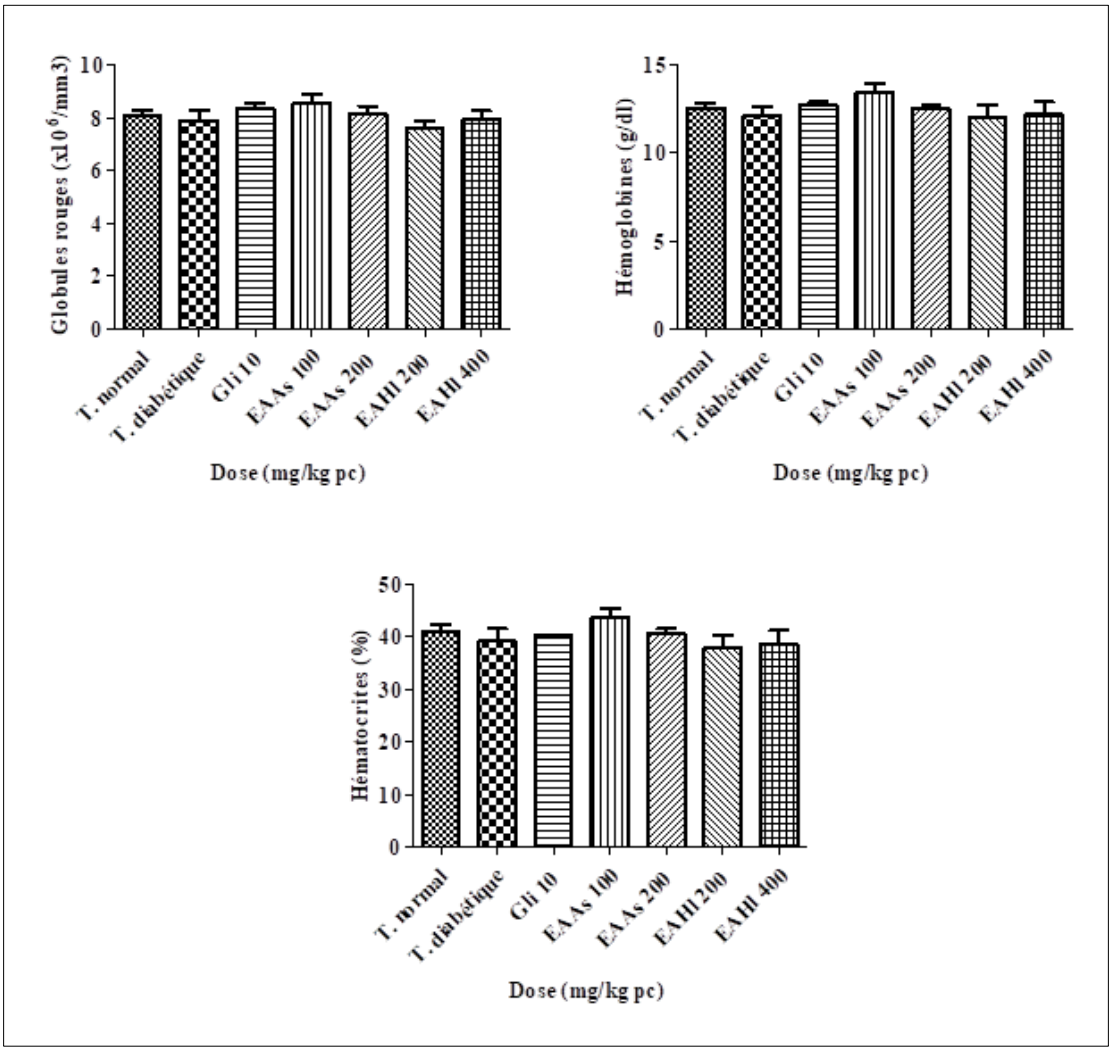

Figure 1 Effects of aqueous extracts of *Annona senegalensis* (EAAs), *Hallea ledermannii* (EAHl) and glibenclamide (Gli) on red blood cells, hemoglobins and hematocrit after four (4) weeks of treatment in diabetic rats.

Results are presented as mean ± SEM, n = 5.

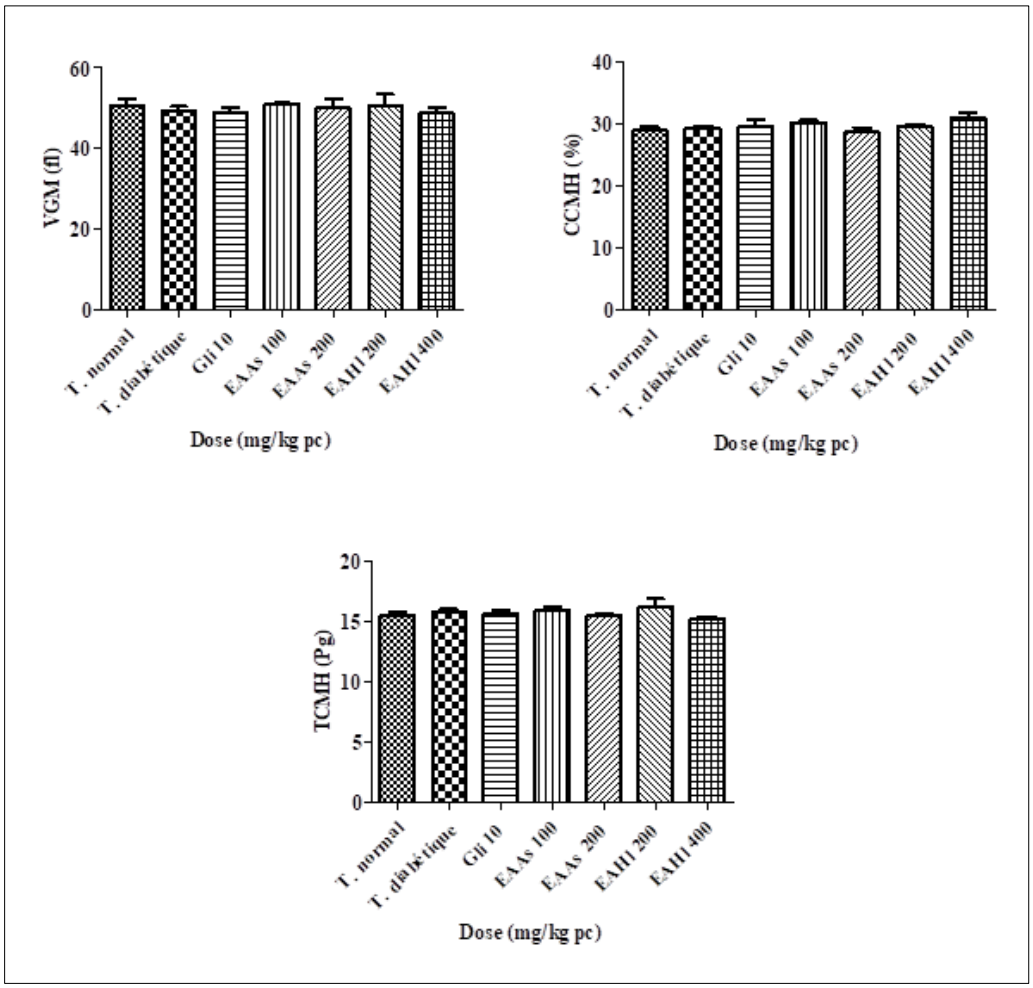

Figure 2 Effects of aqueous extracts of *Annona senegalensis* (EAAs), *Hallea ledermannii* (EAHl) and glibenclamide (Gli) on erythrocyte indices after four (4) weeks of treatment in diabetic rats.

Results are presented as mean ± SEM, n = 5.

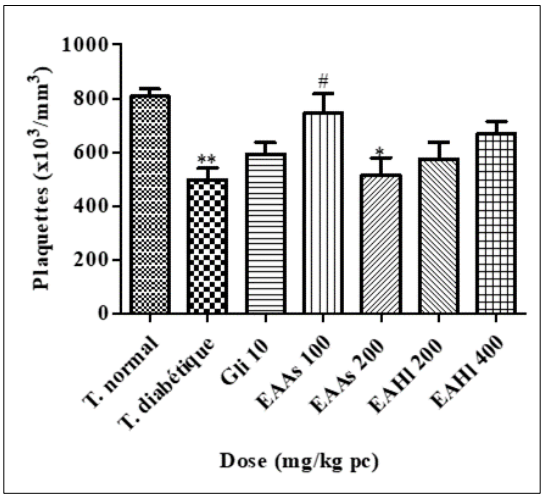

Figure 3 Effects of aqueous extracts of *Annona senegalensis* (EAAs), *Hallea ledermannii* (EAHl) and glibenclamide (Gli) on blood platelets after four (4) weeks of treatment in rats rendered diabetic.

Results are presented as mean ± SEM, n = 5; *p < 0.05;**p< 0.01 compared with non-diabetic controls, #p<0.05 compared with untreated diabetic controls.

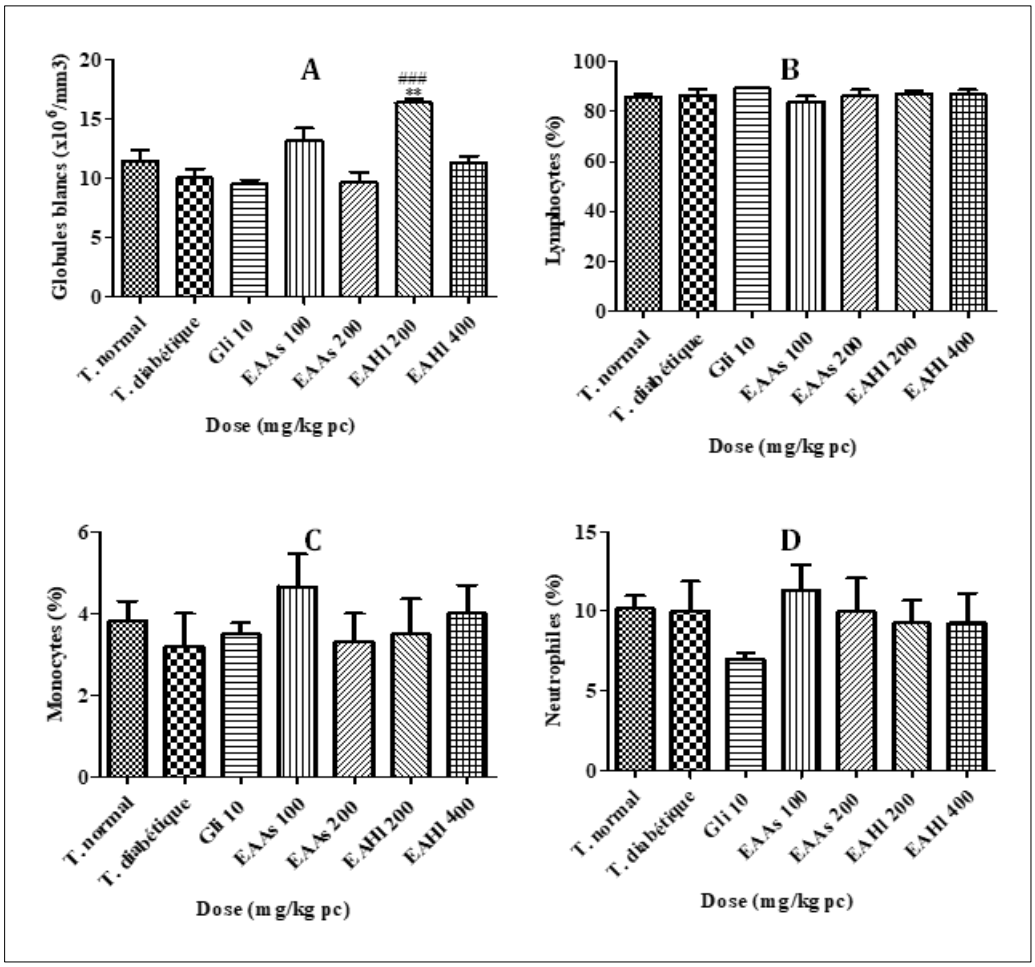

Figure 4 Effects of aqueous extracts of *Annona senegalensis* (EAAs), *Hallea ledermannii* (EAHl) and glibenclamide (Gli) on white blood cells and leukocyte count after four (4) weeks of treatment in diabetic rats.

Results are presented as mean ± SEM, n = 5;

4.2. Effects of extracts after thirteen (13) weeks of treatment

Figure 5 shows the effect of the different substances on red blood cell, hemoglobin and hematocrit content, while figure 6 presents the effect of these substances on erythrocyte indices. These results show that these parameters are not statistically different ($p > 0.05$) in diabetic rats (treated or untreated) compared with values in non-diabetic rats.

In diabetic rats treated with EAHl200, blood platelet content was significantly reduced by 52.71%, compared with non-diabetic controls. At a dose of 400 mg/kg bw EAHl, an increase ($p < 0.05$) of 84.32% in platelets was observed, compared with untreated diabetic controls (Figure 7).

The white blood cell count did not vary significantly ($p > 0.05$) in diabetic rats (treated or untreated) compared to the value of this parameter in non-diabetic animals (Figure 8 A).

In terms of leukocyte count, a decrease in the proportion of neutrophils (Figure 8 D) was observed in some batches of diabetic rats (treated or untreated) compared with non-diabetic rats. This parameter was statistically reduced by 26.98% ($p < 0.05$), by

34.92%, 53.97%, 49.21% and 53.97% respectively in untreated diabetic rats ($p < 0.05$) and in those treated with Gli10 ($p < 0.01$), EAAs100 ($p < 0.001$), EAAs200 ($p < 0.01$) and EAHl400 ($p < 0.01$). When neutrophil counts were compared between treated and untreated diabetic rats, a significant decrease ($p < 0.05$) in this parameter was observed in those treated with EAAs100 and EAHl400.

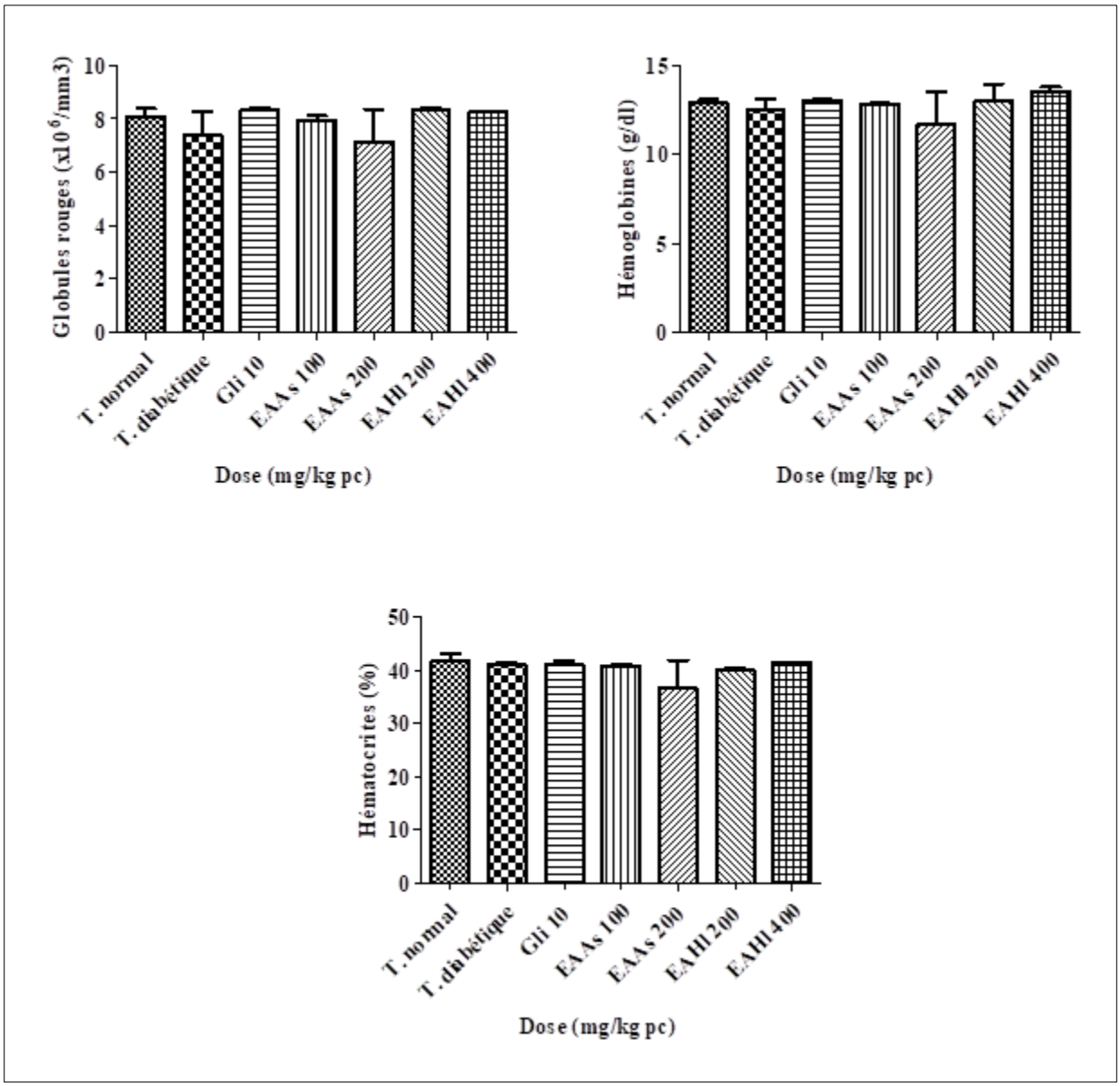

Figure 5 Effects of aqueous extracts of *Annona senegalensis* (EAAs), *Hallea ledermannii* (EAHl) and glibenclamide (Gli) on red blood cells, hemoglobins and hematocrit after 13 weeks of treatment in diabetic rats.

Results are presented as mean ± SEM, n = 5.

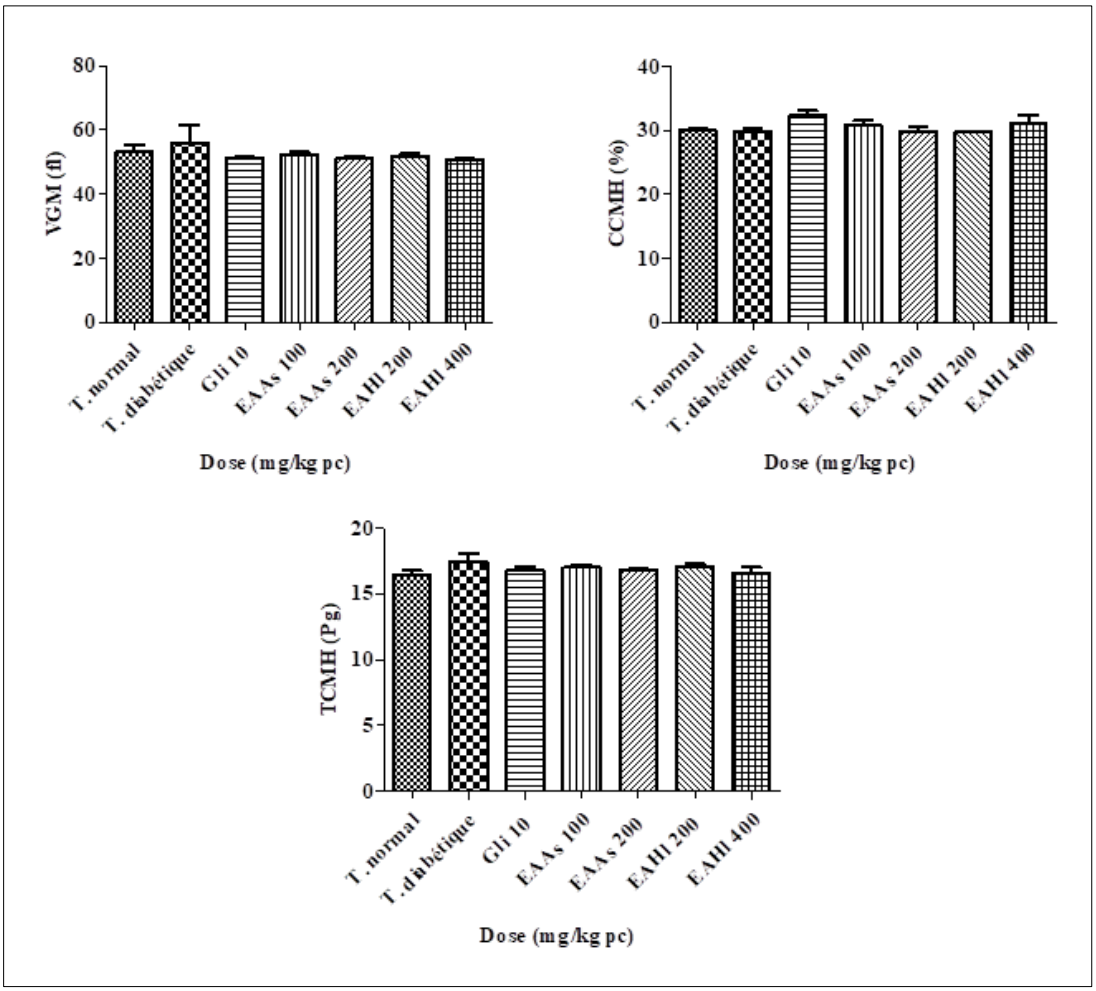

Figure 6 Effects of aqueous extracts of *Annona senegalensis* (EAAs), *Hallea ledermannii* (EAHl) and glibenclamide (Gli) on erythrocyte indices after thirteen (13) weeks of treatment in diabetic rats.

Results are presented as mean ± SEM, n = 5.

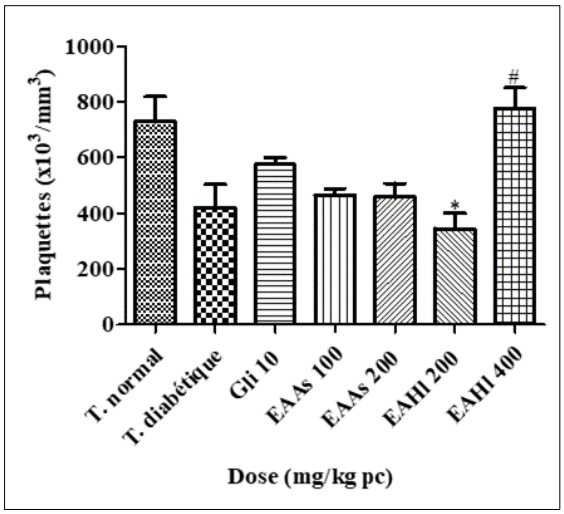

Figure 7 Effects of aqueous extracts of *Annona senegalensis* (EAAs), *Hallea ledermannii* (EAHl) and glibenclamide (Gli) on blood platelets after thirteen (13) weeks of treatment in diabetic rats.

Les résultats sont présentés en moyenne ± SEM, n = 5; *p < 0,05 comparé aux témoins non diabétiques, #p < 0,05 comparé aux témoins diabétiques non traités.

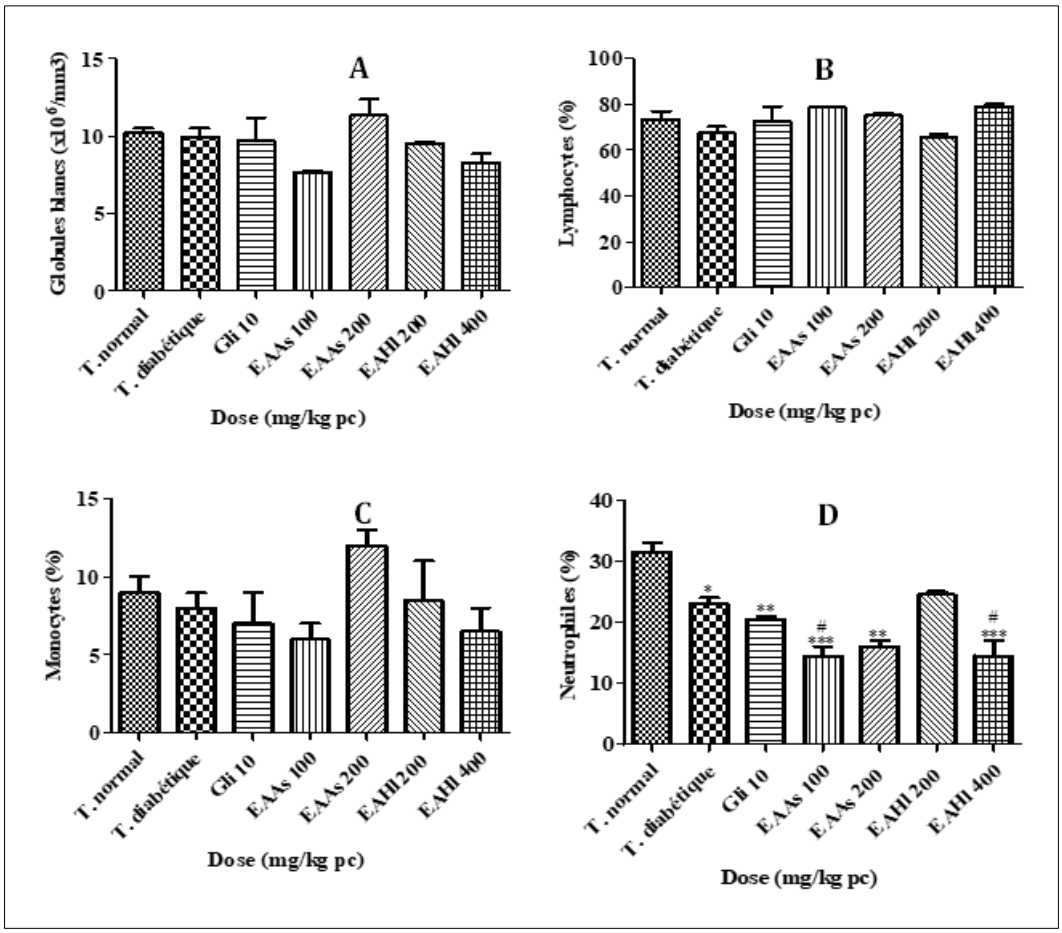

Figure 8 Effects of aqueous extracts of *Annona senegalensis* (EAAs), *Hallea ledermannii* (EAHl) and glibenclamide (Gli) on white blood cells and leukocyte formula after thirteen (13) weeks of treatment in rats rendered diabetic.

Results are presented as mean ± SEM, n = 5; *p < 0.05; **p < 0.01; ***p< 0.001, compared with non-diabetic controls, #p < 0.05 compared with untreated diabeticcontrols.

5. Discussion

To assess the effects of aqueous extracts of *Annona senegalensis* (EAAs) and *Hallea ledermannii* (EAHl) on hematological parameters in rats, blood counts and leukocyte counts were performed after 4 and 13 weeks of treatment.

After four (4) weeks of experimentation, a decrease in platelet count was observed in both untreated diabetics and those treated with EAAs 200, compared with non-diabetic controls.

Our results are similar to those of Keskin et al. (2016). These authors demonstrated a decrease in blood platelet content in diabetic rats treated with quercetin, which would indicate a suppression of hemopoiesis. Platelets are sentinel cells that make a significant contribution to anti-infectious immunity (Chabert et al., 2017).

Hence the drop in their levels in our work shows that EAAs and EAHl have not yet acquired their anti-infectious effect after this two-week experimental period.

In four (4) weeks of experimentation, administration of EAHl 200 resulted in an increase in white blood cells, compared with values in non-diabetic controls and untreated diabetic controls. Keskin et al (2016) obtained similar results in diabetic rats treated with quercetin.

According to some authors, this increase is due to cytokine release (Hofmann et al., 1998; Shanmugam et al., 2003; Kanter et al., 2007; Mahmoud, 2013). This increase in white blood cell content is also thought to be linked to activation by advanced glycation products and oxidative stress in diabetes (Pertynska-Marczewska et al., 2004).

The increase in white blood cell count is thought to be due to the stimulation of the immune system by our plant extracts, which promotes the production of immune cells.

On the 91st day of treatment, a decrease in neutrophils in diabetic animals was observed. (traités ou non) a été observée comparée à la valeur des témoins non diabétiques.

Authors such as Nwankpa et al (2014) obtained similar results in Wistar rats infested with *Salmonella typhi (Enterobacteriaceae)* and treated with *Phyllanthus amarus* leaf extract *(Phyllanthaceae).* The authors explained this reduction by the fact that neutrophils involved in phagocytosis of foreign chemicals in the body are subsequently destroyed.

Plant extracts have also been shown to inhibit the growth of certain human pathogens (Agrawal et al., 2004; Notka et al., 2004).

Our results are contrary to those of some authors whose work has focused on haematological parameters in rats (Aka et al., 2016; Nene Bi al., 2016). Indeed, Nene Bi et al. (2016) showed that daily administration over a period of 90 days of the aqueous extract of *Bridelia ferruginea (Euphorbiaceae)* did not lead to changes in haematological parameters in male rats.

Similarly, according to Aka et al. (2016) administration of aqueous extract of *Coffea canephora (Rubiaceae)* at 200 mg/kg bw to diabetic rats did not cause any significant variation in platelet count and leukocyte formula after ninety-one (91) days of experimentation.

6. Conclusion

The evaluation of hematological parameters is used to determine the adverse effect of foreign compounds, plant extracts on the blood components of animals. This study shows a decrease in red blood cells, platelets and neutrophils due to the anti-oxidant effect of the aqueous extracts of *Annona senegalensis* and *Hallea ledermannii* used, and their anti-infectant properties.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Prior to this work, the acute toxicity test for the plants used, in Wistar rats, was carried out in accordance with the guidelines of the Organisation for Economic Co-operation and Development (OECD 423 of 2001).

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