

Biological effect of the Total Aqueous Extract of *Desmodium Adscendens* Leaves, on Blood Cells after Inducing Hepatotoxicity in Rats

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Abstract: The antihepatotoxicity effects of the total aqueous extract of the leaves of *Desmodium adscendens* were evaluated at doses of 100, 200 and 400 mg/kg BW on the number of blood cells in the model of hepatitis induction with paracetamol in rats. Six groups of six rats are constituted. The group 1 received distilled water; the group 2, the paracetamol 200 mg/kg B; the group 3, the paracetamol 200 mg/kg BW and vitamin C 100 mg/kg BW; the group 4, paracetamol 200 mg/kg BW and the extract 100 mg/kg BW; the group 5, the paracetamol 200 mg/kg BW and the extract 200 mg/kg BW and the group 6, the paracetamol 200 mg/kg BW and the extract 400 mg/kg BW. The solutions were administered by gavage for 30 days, the first fortnight for the induction of hepatitis and the second fortnight for its treatment. Blood samples were taken on the 2nd, 17th and 32nd days.

The results show that the total aqueous extract and vitamin C lead to a dose-dependent and significant inhibition of the action of paracetamol. The total aqueous extract normalizes and stabilizes the number of defense blood cells.

These studies showed the hepatoprotective through a stabilization of the number of blood cells by the total aqueous extract of *Desmodium adscendens* leaves.

Keywords: Blood cells, *Desmodium adscendens*, Hepatitis, Paracetamol.

1. INTRODUCTION

Hepatitis has spread widely throughout the world in general and in particular in Côte d'Ivoire, where the prevalence of hepatitis C is 3.3% in the general population according to [1]. According to [2], The prevalence of hepatitis B is 12% in Côte d'Ivoire. Despite these alarming figures, treatment for hepatitis B and C remains very limited [3].

Obviously, hepatitis causes the body to react by mobilizing defense blood cells like any other pathology. Modern drugs struggle to satisfy the treatment of hepatitis especially to normalize and organize blood cells for good defense of the body. Thus, traditional medicine has become an important alternative for populations who lack financial means. Medicinal plants are widely used in the prevention and treatment of various pathologies in Africa and in developing countries, and are today sources of natural substances used in the treatment of several diseases [4]. *Desmodium adscendens* which is the subject of our study, is a plant commonly used in the traditional treatment of inflammations such as hepatitis by Ivorian populations mainly from the center-west, through its leaves. Despite this constant use by our populations, the plant has not yet been the subject of scientific studies to show its effect on blood cells after inducing hepatitis. This work aims to promote the biodiversity of local flora through the therapy of pathologies. This study aims to provide also a scientific basis for the traditional use of this plant, by evaluating the effect on blood cells, of the

aqueous extract of *Desmodium adscendens* leaves in rats suffering from hepatitis linked to drug poisoning, with paracetamol.

2. MATERIAL AND METHODS

Material

Plant material

The plant material is constituted of the leaves of *Desmodium adscendens*. The harvest period for the leaves of this plant species took place in the month of October 2022 at Daloa, a city located in the center-west of Ivory Coast. The species was identified at Nangui Abrogoua University in Abidjan, at the Laboratory of Physiology, Pharmacology and Pharmacopoeia (LPPP).

Animal material

The rats used in these experiments are male and female albino rats of the *Rattus norvegicus* species. These adult rats are eight (8) weeks old, with a body mass between 156 and 211 g. These rats are acclimated to the ambient temperature of $25\pm 3^{\circ}\text{C}$ for 05 days before the start of the manipulation. These animals are fed with pellets from the company FACI®. The tap water used to water them in bottles was constantly renewed. These animals are come from the Laboratory of Physiology, Pharmacology and Pharmacopoeia of Nangui Abrogoua University. The different experimental protocols are followed in accordance with the protocols for the protection of laboratory animals of the European Council of Legislation 87/609/EEC [5].

Methods

Preparation of the aqueous extract of the leaves of *Desmodium adscendens*

The extraction procedure described by [6] was used to prepare the total aqueous extract of *Desmodium adscendens* leaves. The leaves of *Desmodium adscendens* were harvested, washed and dried away from the sun. After drying, they were crushed and reduced to a fine powder using an electric grinder. One hundred (100) grams of this powder were extracted in one liter of distilled water by decoction for 15 minutes. The decoction obtained was filtered through cotton then through Whatman filter paper; The residue obtained was taken again in a liter of distilled water and also subjected to a decoction for 15 minutes. The filtrate obtained was evaporated and dried in an oven at 50°C . We obtain a blackish green powder which will make it possible to prepare the total aqueous extract of the leaves of *Desmodium adscendens* for studies on blood cells after inducing hepatitis.

Preparation of paracetamol solution

Preliminary studies were carried out to obtain the toxic dose of paracetamol. Paracetamol tablets (Doliprane® 1000mg (SANOFI)) are made into powder and dissolved in distilled water then administered to rats by gavage to determine the LD_{50} . At a dose of 500 mg/kg BW, half of the animals died during the experiment. The dose of 200 mg/kg BW was therefore used for 15 days to induce hepatotoxicity in rats [7].

Preparation of vitamin C solution

This solution is obtained according to the method used during the work of [8]. These authors showed that vitamin C at a dose of 100 mg/kg of PC had a hepatoprotective effect in rats.

Preparation of doses of the total aqueous extract of *Desmodium adscendens* leaves

The different doses of the total aqueous extract of the leaves of *Desmodium adscendens* were prepared according to that recommended by traditional therapists for an adult man per day. Thus, the different doses of 100, 200 and 400 mg/kg BW were prepared to carry out the studies.

Solution administration

The volume of substance to be administered to a 100 g rat is 1 or 2 mL according to OCDE protocol 407 [9].

Experimentation

Hepatitis induction method with paracetamol

The studies are carried out according to the methods of Dougnon et al. (2009). Thirty-six (36) rats are divided into six (6) groups, each comprising three (3) males and three (3) females and the solutions will be administered orally with the feeding tube:

- Group1: They have access to water and food; the animals will not receive any treatment,

- Group2: The rats receive 1 mL of the solution of paracetamol(Doliprane®) 200 mg / kg BW for two weeks,
- Group 3: The animals receive 1 mL of the solution of 200 mg / kg BW of paracetamol (Doliprane®) per day for two weeks, then 2 mL of the solution of 100 mg / kg BW of vitamin C for two weeks,
- Group4: The rats received 1 mL of the solution of 100 mg/kg BW of paracetamol (Doliprane®) for two weeks, then 1 ml of the solution of 100 mg/kg BW of the total aqueous extract of the leaves of *Desmodium adscendens* for two weeks,
- Group 5: The rats receive 1 ml of the solution of 200 mg/kg BW of paracetamol (Doliprane®)for two weeks, then 1 ml of the solution of 200 mg/kg BW of the total aqueous extract of the leaves of *Desmodium adscendens* for two weeks,
- Group 6: The rats received 1 ml of the solution of 200 mg/kg BW of paracetamol (Doliprane®) for two weeks then 1 mL of the solution of 400 mg/kg BW of the total aqueous extract of the leaves of *Desmodium adscendens* for two weeks.

Every two days, new solutions were prepared taking into account the new weight acquired per animal per group, until the end of the experiment. The first blood samples are taken on day 2 in the animals before the induction of hepatitis. The second blood samples are taken on day 17 after induction of hepatitis with paracetamol for two weeks in groups 2, 3, 4, 5 and 6. The last samples are taken on day 32 after treatment with vitamin C or the total aqueous extract of the leaves of *Desmodium adscendens* for two weeks in groups 3, 4, 5 and 6.

Blood sample

At the end of the treatment and after fasting for 12 hours, the animals are anesthetized with ether. Blood is collected from the retro-orbital sinus using sterile syringes for the measurement of blood cells according to the method of [10].The EDTA tube was used to collect whole blood in order to carry out the blood cell count.

Statistical analysis

In pharmacological experiments, results are expressed as means \pm SEM (Standard Error of Mean). The analysis of variance test (ANOVA 1) will be used to determine the statistical significance of the results ($p < 0.05$). Graphpad Prism 8 Demo and Excel software are used to carry out these statistical tests and graphs.

3. RESULTS

Effect of the aqueous extract of *Desmodium adscendens* leaves on blood cells in rats renderedhepatic by paracetamol

Dosage on the day 2

Dosage of red blood cells (RBC), white blood cells (WBC) and lymphocytes (LPT)

Normal levels of red blood cells, white blood cells and lymphocytes are between 4.26 ± 0.67 and 5.93 ± 0.67 $10^6/uL$, respectively; between 1.78 ± 0.2 and 3.83 ± 1.73 $10^3/uL$ and between 1.23 ± 0.9 and 1.85 ± 0.4 $10^3/uL$ (figure 1).

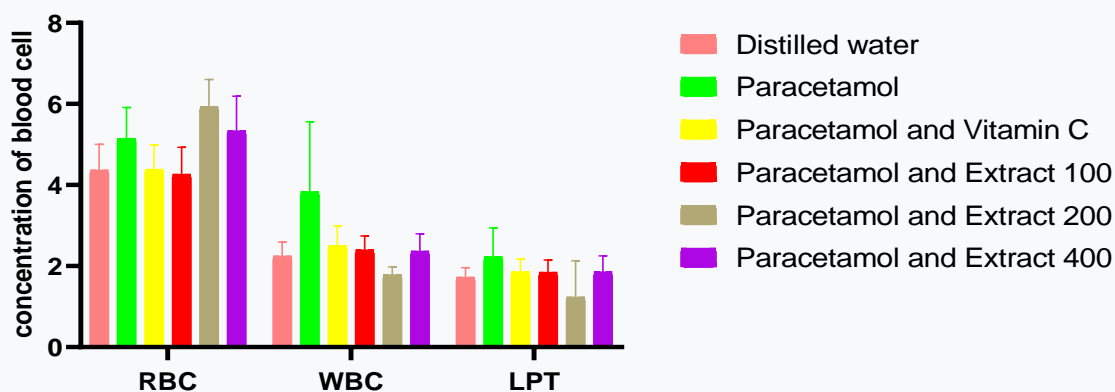


Figure1. Evolution of red blood cell, white blood cell and lymphocyte on Day 2

Dosage of blood platelets (BP)

Normal blood platelets levels are between 431.1 ± 11 and 726.8 ± 12 103/uL, respectively (Figure 2).



Figure2. Evolution of blood platelets on Day 2

Dosage on day 17

Dosage of red blood cells (RBC), white blood cells (WBC) and lymphocytes (LPT)

The number of red blood cells increased and reached a maximum of 12.9 ± 1.2 10⁶/uL in rats receiving paracetamol only. The level of white blood cells also rises and reaches a maximum of 7.4 ± 1.5 10³/uL in the batch of rats which will be treated with the extract at a dose of 400 mg/kg BW. The level of lymphocytes increases and reaches 5.4 ± 1.3 10³/uL in the group which receives only paracetamol (figure 3).

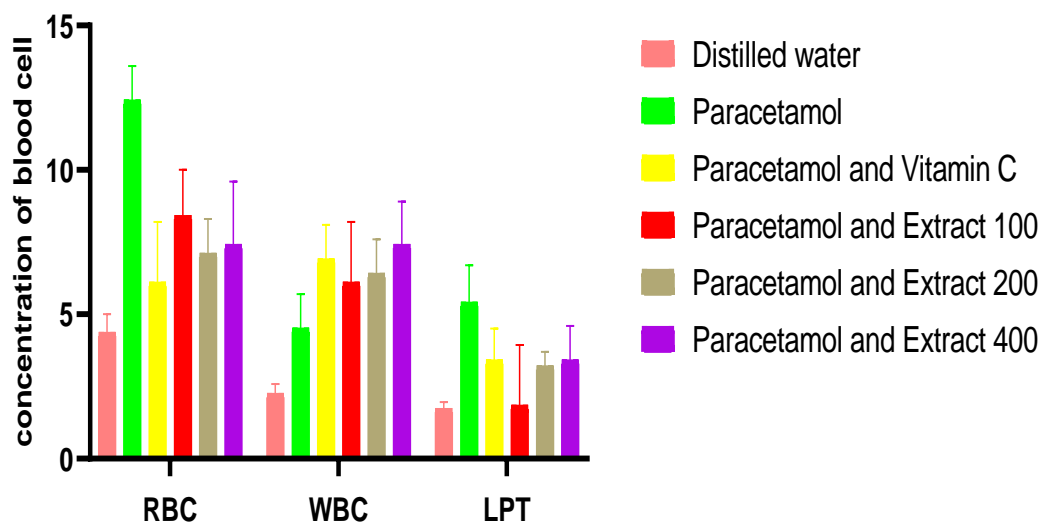


Figure3. Evolution of red blood cell, white blood cell and lymphocyte on Day 17

Dosage of blood platelets (BP)

After overdose with paracetamol, the level of blood platelets decreases and reaches a minimum of 321.2 ± 9 ,103/uL in rats which will receive only paracetamol (figure 4).

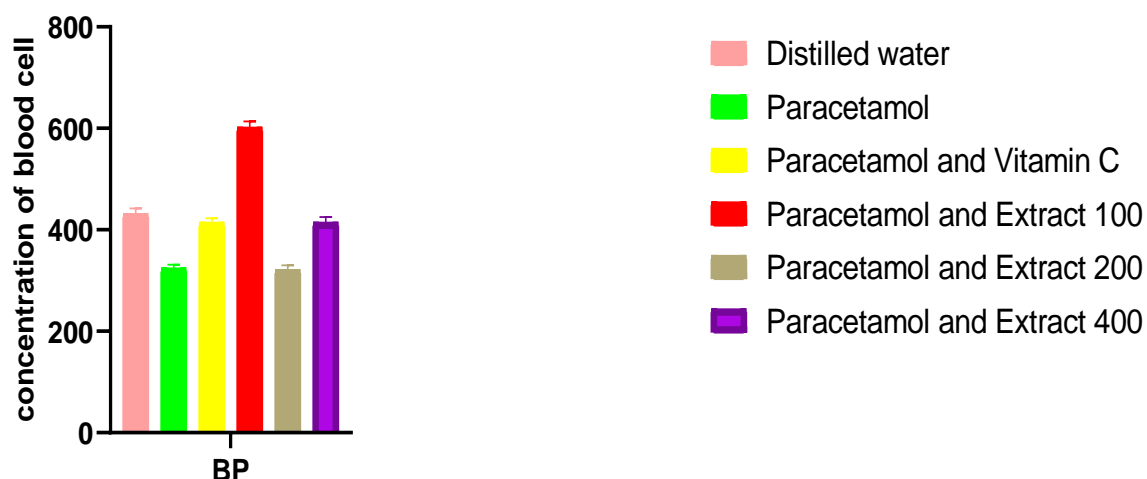


Figure4. Evolution of blood platelets on Day 17

Dosage on day 32

Dosage of red blood cells(RBC), white blood cells(WBC) and lymphocytes(LPT)

The aqueous extract and the vitamin C normalize the levels of defense blood cells. They return the levels to normal after neutralization of hepatitis. Thus, the aqueous extract reduces the red blood cell count from 12.4 ± 1.2 to 1.5 ± 1.2 $10^6/uL$ with vitamin C and to 1.6 ± 1.3 $10^6/uL$ with aqueous extract at a dose of 400 mg/kg BW. The aqueous extract at a dose of 400 mg/kg BW and vitamin C normalizes the white blood cell level to 1.5 ± 1.4 $10^3/uL$ and 1.9 ± 1.4 $10^3/uL$ respectively compared to the batch. control which is 4.5 ± 1.2 $10^3/uL$. The lymphocyte level is reduced and stabilized at 1.4 ± 0.2 $10^3/uL$ with the aqueous extract at the maximum dose of 400 mg/kg BW and at 1.2 ± 0.2 $10^3/uL$ with vitamin C (figure 5).

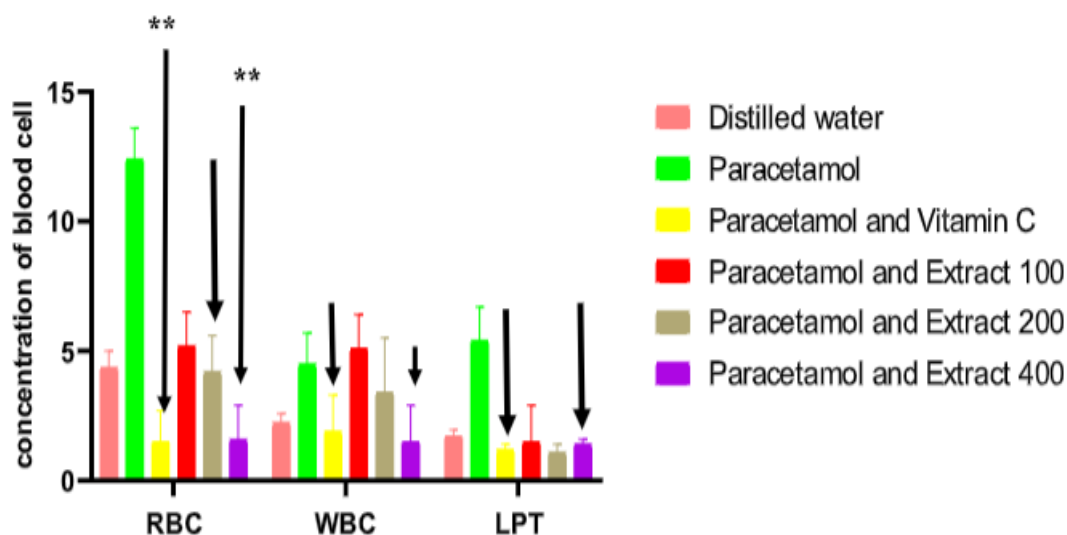


Figure5. Evolution of red blood cell, white blood cell and lymphocyte on Day 32

Results are expressed as Means \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Dosage of blood platelets (BP)

The blood platelet level increased significantly to 56.62% (from 324 ± 7.2 to 714 ± 8.4 $10^3/uL$) for the batch treated with vitamin C and 52.92% (from 324 ± 7.2 to 688.2 ± 10.1 $10^3/uL$) for the batch which received the extract at the maximum dose of 400 mg/kg BW (figure 6).

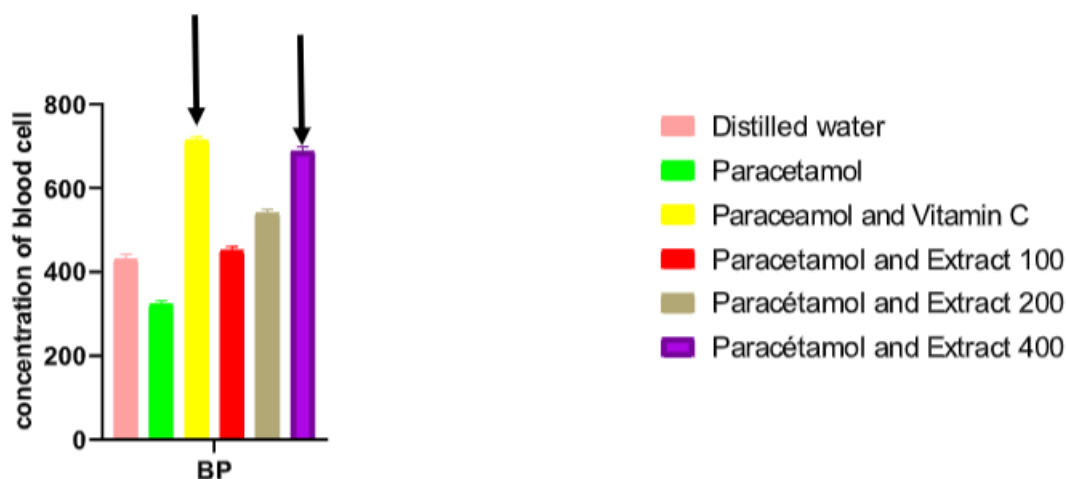


Figure6. Evolution of blood platelets on Day 32

Results are expressed as Means \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4. DISCUSSION

Our results revealed normal values of blood cell counts in rats. Normal levels of red blood cells, white blood cells and lymphocytes are between 4.26 ± 0.67 and 5.93 ± 0.67 $10^6/\mu\text{L}$, respectively; between 1.78 ± 0.2 and 3.83 ± 1.73 $10^3/\mu\text{L}$ and between 1.23 ± 0.9 and 1.85 ± 0.4 $10^3/\mu\text{L}$. These results are significantly close to those obtained by [11].

Paracetamol overdose has a double toxic action: on the one hand on liver cells through necrosis and on the other hand on cells blood cells [12]. The mechanism of liver intoxication by paracetamol essentially targets parenchyma cells: which explains the increase in transaminases and blood sugar according to [13]. Paracetamol is metabolized by cytochrome P 450 to give an active metabolite N-Acetyl-P-benzoQuinone-Imine (NAPQI) leading to hepatic necrosis by peroxidation of membrane lipids (release of peroxy radicals) subsequently causing lysis cellular [7]. Also, according to these latter authors, liver cells have glutathione for their defense, which constitutes their main antioxidant. Paracetamol overdose through the active metabolite NAPQI causes liver depletion of glutathione [14]. This depletion is due to the excessive consumption of glutathione, the immediate consequence of which is lipid peroxidation [15] and oxidation of the thiol groups of proteins [16].

Our results showed an increase in lymphocytes and white blood cells after inducing hepatitis. These observations are identical to those of [17] who revealed an increase in these parameters following liver infection. Studies have also shown that during liver poisoning, the concentration of blood cells such as lymphocytes and white blood cells increase in the blood to strengthen the body's immunity [12]. Our studies also showed a reduction in blood platelets. According to [12], the liver is responsible for the production of most of the coagulation factors which constitute the main role of blood platelets. An infection therefore of the liver would lead to a drop in blood platelets.

Studies carried out after administration of vitamin C or the aqueous extract showed a significant increase in blood platelets and a normalization of the levels of red blood cells, white blood cells and lymphocytes compared to the control group. These results are consistent with those obtained by [18] who showed a slight increase with stabilization of blood cell levels after administration of the aqueous extract of the mesocarp of the *Garcinia kola* fruit. Similar results were also obtained by [19] with the extract of *Ficus sycomorus* leaves in rats after inducing hepatitis. Therapeutic phytochemical constituents found in the leaves of *Desmodium adscendens* are composed of acids, alkaloids, flavonoids, polyphenols and anthocyanins [20]. [21] showed that fatty acids cleansed and purified the blood by renewing the body's defense cells. The flavonoids have a profound impact on several immune cells including T cells, B cells, NK cells and immune mechanisms that are important in inflammatory processes [22]. Polyphenols allow the modulation of immune functions [23]. The increase in the level of blood platelets caused by the aqueous extract could be due to the fact that the latter are sentinel cells and contribute significantly to anti-infectious immunity [24].

5. CONCLUSION

The results of this study showed that the aqueous extract of *Desmodium adscendens* leaves had immunostabilizing activity. To this end, it normalizes and stabilizes the number of blood cells disrupted by paracetamol intoxication. The phytochemical constituents contained in the aqueous extract of the leaves could be responsible for the effects observed on the blood cell count.

Abbreviations

BW : Body weight

UE : European Union

OCDE : Organisation for Economic Co-operation and Development

Competing interests

The authors declare that there are no competing interests.

Authors' Contributions: K.D; development of the methodology and execution of the manipulation. K.D.D; obtaining handling products and participating in handling. G.A.O; processing of results and creation of graphs. All authors have read and approved the final manuscript.

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