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# Acute and subacute toxicity studies of hydroethanolic extract of *Baillonella toxisperma* Pierre fruit pulp

**Takuissu Nguemto Guy Roussel[1,2,3*], Edoun Ebouel Ferdinand Lanvin[1,3], Youovop Fotso Janvier Aimé[1], Ngondi Judith Laure[1] and Oben Julius Enyong[1,2]**

[1]Department of Biochemistry, Laboratory of Nutrition and Nutritional Biochemistry, P. O. Box 812, Faculty of Science, University of Yaounde I, Cameroon.
[2]Cameroon Nutrition and Dietetics Research Center, J&A Oben Foundation, P. O. Box 8348, Yaounde, Cameroon.
[3]Institute of Medical Research and Studies of Medicinal Plants (IMPM), P. O. Box: 13 033, Yaounde, Cameroon.

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***Baillonella toxisperma* Pierre, fruit pulp is largely used in traditional medicine. No toxicity study was done on this fruit pulp. The purpose of this study was to assess the acute and subacute toxicity of the hydroethanolic extract of the fruit pulp of *B. toxisperma*. An acute toxicity study was conducted at 2000 mg/kg of body weight as a 14-day limit test in Wistar albino rats. Throughout the experiment, general signs of toxicity were noted. Subsequently, a subacute toxicity study was performed at a dose of 400 mg/kg of body weight for 28 days. General signs of toxicity were also noted, after animal sacrifice, histological, hematological, and biochemical analyses (ALAT, ASAT, triglycerides, total cholesterol, total proteins, creatinine) were performed. At $LD_{50}$>2000 mg/kg, no general signs of toxicity were noted in either acute or subacute conditions. No signs of toxicity were noted histologically, while slight increases in granulocytes and decreases in lymphocytes were noted hematologically. Biochemically, while no renal observations were made, the extract caused hepatic cytolysis. The fruit pulp of *B. toxisperma* P could be hepatotoxic at high doses.**

**Key words:** Hydroethanolic extract of *Baillonella toxisperma* Pierre fruit pulp, acute toxicity, subacute toxicity.

## INTRODUCTION

Many drugs have been developed for the management of infectious diseases, and those related to poor nutrition. However, these drugs are sometimes not available to many populations because of the generally high cost; combined with this, many side effects call into question the safety of these drugs. This, as well as many populations and even many studies, are increasingly focused on plants as a good source of bioactive compounds with medicinal attributes and known for their low toxicity (Akshada et al., 2019). According to WHO, about 80% of the world's population uses medicinal plants (WHO, 2013). Further evidence of the growing interest in these plants, more than half of the approved drugs were made from plants (Chavan et al., 2018). However, although many medicinal plants are widely consumed, they are potentially toxic (Frenzel and Teschke, 2016). Some of the bioactive compounds in plants affect the entire body, while others affect only a specific organ, such as cardiac glycosides, which are hepatotoxic (Bandara et al., 2010). These toxic effects

*Corresponding author. E-mail: tgroussel@yahoo.fr.

depend on the condition of the plant, the stage of development, the part of the plant used, the species, the amount consumed, and the vulnerability of the organism (Botha and Penrith, 2008). Toxicology, therefore, becomes an important part of pharmacology insofar as it is important to know the adverse effects of phytocompounds on living organisms before their possible clinical use (Sharma et al., 2021). Toxicity studies, therefore, make it possible to make decisions on whether or not to use drugs clinically (OECD, 2008).

*Baillonella toxisperma* Pierre (Sapotaceae), is mainly exploited for its wood; its oil is also commonly sold (Doucet and Kouadio, 2007). Its bark is traditionally used to treat abscesses, stomach pain, rheumatism, infertility, convulsions, and malaria (Ntie-Kang et al., 2013). Fungoh *e*t al. (2015) found that the high polyphenol content of fruits, sometimes responsible for toxic effects (Bandara et al., 2010). Its membership in the family of Sapotaceae, which are latex plants, may suggest possible toxicity. Indeed, Bafor et al. (2020), Adebayo et al. (2010) had already shown the toxic effects of methanolic extract of *Omphalocarpum procerum* sterm bark and ethanolic leaf extract of *Chrysophyllum albidum* respectively. Despite the large use of the extract of *B. toxisperma* in traditional medicine, there is a lack of studies related to their toxicity. The purpose of this study was to evaluate the acute and subacute toxicity of hydroethanolic extract from *B. toxisperma* fruits.

## MATERIALS AND METHODS

### Chemicals

All the chemicals used were of analytical grade and were purchased from Sigma Co., Louis, MO, USA.

### Plant material

Mature fruits of *B. toxisperma* Pierre were harvested in Ondodo (East-Cameroon). They were identified at the Cameroon National Herbarium (N°. 54060/HNC) in Yaounde, where voucher specimens have been deposited under the reference number.

### Experimental animals

Adult male Wistar albino rats weighing 180-230 g were obtained from the animal house of the Department of Biochemistry, University of Yaounde I, Cameroon. The animals were acclimatized in the experimental animal room for 7 days with a 12 h light and 12h dark cycle before the start of experimentation. Standard feed and water were provided *ad libitum* to all experimental animals.

### Extraction

After drying in an oven at 50°C for 3 days, the pulp of *B. toxisperma* Pierre fruit was separated from the kernel. The pulp (100 g) was ground and extracted by maceration for 48 h with 800 ml of solvent (hydroethanol (1:1, v/v). The resulting supernatant was filtered using Whatman N°. 1 filter paper (Whatman International Limited, Kent, England) and concentrated to about 10% of the original volume by a rotavapor before drying in an oven at 50°C to obtain the hydroethanolic extract.

### Oral acute toxicity study

The acute oral toxicity study was sanctioned to be conducted in compliance with OECD guideline 423, which stipulates the use of only three animals per group (OECD 423, Paragraph 23) (Jonsson et al., 2013; Sankhari et al., 2010). The animals were fasted overnight (~12 h) and weighed. Two groups of 3 rats each were used. One of these groups was treated with the extract and the second was administered with the vehicle (water). A test dose of extract was calculated concerning the body weight of every fasted animal and administered via oral gavage at 2000 mg/kg of body weight. The animals were regularly and individually observed for behavioral changes (motricity, weakness, aggressivity, audition and pain sensibility) and general toxicity signs (body weight loss, change in skin, diarrhea, rate of respiration, coma, and death) after dosing for the first 24 h, with special attention being given during the first 4 h. Thereafter, observation was continued daily for a total of 14 days (Nana et al., 2011).

### Experimental design of oral subacute toxicity study

For this study, the rats, numbering 8, were divided into 2 groups of 4 each, according to the OECD 407 guideline (OECD, 2008). One of these groups was treated with the extract (400 mg/kg of body weight) and the second was administered with the vehicle (water). The weights of rats were noted every week, while the behavioral changes (motricity, weakness, aggressivity, audition, and pain sensibility) and general toxicity signs (body weight loss, change in skin, diarrhea, rate of respiration, coma, and death) were registered every day. The animals were administered by gavage, daily, for 28 consecutive days. At the end of the experiment, the animals were sacrificed to collect their blood and organs for relative weight (heart, liver, pancreas, brain, kidney, lung, spleen, testis, and stomach) biochemical (blood), hematological (blood) and histological (liver, kidney, and spleen) analyzes.
NB: The animal experiments were carried out following the guideline of NIH (1978).

### Blood analysis

Biochemical and hematological analyzes were done. Biochemical analyzes included:

(i) Liver metabolic activity markers: total cholesterol (Roeschlau et al., 1974), triglycerides (Fossati and Principe, 1982) and total proteins (Lowry et al., 1951),
(ii) Hepatic cytolysis markers: ASAT and ALAT (Reitman and Frankel, 1957),
(iii) Nephrotic markers: Urea and creatinine (Bartels et al., 1972).
Hematologic parameters included red and white blood cells, hemoglobin, hematocrit, MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), MPV (Mean Platelets Volume), platelets, lymphocytes, monocytes, and granulocytes, with the unit XN1000 (Sysmex).

### Histopathological examinations

For all autopsied animals, the tissues or organs (liver, kidney, and spleen) were removed and weighed. The samples were fixed in

**Table 1.** General appearance and behavioral observations of acute toxicity study.

| Observation | Normal group | Test group |
|---|---|---|
| Motricity | Normal | Normal |
| Weakness | Not present | Not present |
| Aggressivity | Not present | Not present |
| Audition | Normal | Normal |
| Pain sensibility | Normal | Normal |
| Body weight | Not lost | Not lost |
| Change in skin | No effect | No effect |
| Diarrhea | Not present | Not present |
| Rate of respiration | Normal | Normal |
| Coma | Not present | Not present |
| Death | Alive | Alive |

**Table 2.** General appearance and behavioral observations of subacute toxicity study.

| Observation | Normal group | Test group |
|---|---|---|
| Motricity | Normal | Normal |
| Weakness | Not present | Not present |
| Aggressivity | Not present | Not present |
| Audition | Normal | Normal |
| Pain sensibility | Normal | Normal |
| Body weight | Not lost | Not lost |
| Change in skin | No effect | No effect |
| Diarrhea | Not present | Not present |
| Rate of respiration | Normal | Normal |
| Coma | Not present | Not present |
| Death | Alive | Alive |

10% natural buffered formalin and then subjected to general tissue treatments such as dehydration and paraffin embedding. Tissue sections were cut and hematoxylin and eosin staining were performed. Histopathological examination under the light microscope was performed on tissues and organs of all individuals in the control and test group.

#### Statistical analysis

All data were expressed as mean values ± standard deviation. Significant differences among the groups were determined by the ANOVA test, following the post hoc of Bonferonni using the SPSS statistical analysis program. Statistical significance was considered at $p<0.05$.

## RESULTS

### Acute toxicity

The oral administration of hydroethanolic extract of the fruit pulp of *B. toxisperma* at 2000 mg/kg induced no abnormal signs of toxicity (Table 1). After administration of the unique dose of extracts, all animals presented similar behavior, no signs of toxicity, and no mortality was registered after 14 days. The extract seems to be safe at a dose level of 2000 mg/kg of body weight, and the $LD_{50}$ was considered to be >2000 mg/kg.

### General signs of subacute toxicity

The oral administration of hydroethanolic extract of the fruit pulp of *B. toxisperma* at 400 mg/kg induced no abnormal signs of toxicity (Table 2). After administration of the daily dose of extracts, all animals presented similar behavior, no signs of toxicity, and no mortality was registered after 28 days.

### Body weight variation

The effects of the extract on the body weight variation

**Table 3.** Body weight variation.

| Time (days) | Weights (g) | |
|---|---|---|
| | Normal group | Test group |
| 0 | 165.75±38.56(0.00%) | 165.00±37.66(0.00%) |
| 7 | 170.00±40.72(2.56%) | 169.50±37.26(2.72%) |
| 14 | 172.75±39.91(4.22%) | 170.50±37.82(3.33%) |
| 21 | 179.25±37.61(8.14%) | 175.75±36.74(6.51%) |
| 28 | 182.25±38.29(9.95%) | 178.25±35.43(8.03%) |

The values are expressed as means ± standard deviations. The values in brackets represent the percentages of weight change. No significant difference at $p<0.05$ was observed, comparison made between values of the same line.

**Table 4.** Relative organ weights.

| Organ | Normal group | Test group |
|---|---|---|
| Liver | 0.027±0.003[a] | 0.023±0.004[a] |
| Kidneys | 0.006±0.0002[a] | 0.006±0.0003[a] |
| Brain | 0.006±0.0006[a] | 0.007±0.0007[a] |
| Lung | 0.006±0.0004[a] | 0.006±0.0007[a] |
| Spleen | 0.004±0.0006[a] | 0.003 ±0.0003[a] |
| Heart | 0.003±0.0002[a] | 0.003±0.0003[a] |
| Stomach | 0.009±0.0004[a] | 0.007±0.0007[b] |
| Pancreas | 0.004±0.0007[a] | 0.003±0.0003[a] |
| Testis | 0.009±0.002[a] | 0.010±0.0004[a] |

The values are expressed as means ± standard deviations. The values assigned different letters on the same line are significantly different at $p<0.05$.

were represented in Table 3. No significant difference was noted between the body weight of the normal and test group.

## Relative organ weights

The effects of the extract on the relative organ weights were represented in Table 4. Excepted in the stomach where we noted atrophy in the test group (0.007) in comparison to the normal group (0.009), no significant difference was noted with the other organs.

## Histological analysis

The results of the study of histological sections of the liver, spleen, and kidney are shown in Figures 1 to 3 respectively. Microphotographs of the liver of control animals and rats treated with hydroethanolic extract of the fruit pulp of *B. toxisperma* show a normal architecture of hepatic parenchyma with hepatocytes arranged in paving stones around the centrilobular vein. The microphotographs of the kidney of the animals in the 2 groups show a normal structural arrangement with a glomerulus, a urinary space, and distinct tubules. Microphotographs of the spleen of animals in the different groups show a normal architecture with white pulp (lymphatic nodule containing an arterial and a germinal center, where lymphocytes and lymphoblasts proliferate) and a red pulp (splenic cord, arteriole, and venous sinus).

## Hematological parameters

The effects of the extract on the hematological parameters were represented in Table 5. To compare with the normal group, the test group showed the highest level of granulocytes (32.4 vs 22.85%) and the lowest level of platelets (204.75 $10^3$ vs 262.5 $10^3$/µl) and lymphocytes (56.1 vs 66.45%). No significant difference was noted with the other hematological parameters.

## Metabolic activities of the liver

The effects of the extract on the metabolic activities of the liver were represented in Table 6. To compare with normal group, test group shown the lowest levels of triglycerides (50.31 vs 59.25 mg/dl) and total cholesterol (66.61 vs 83.39 mg/dl). No significant difference was noted with total proteins.

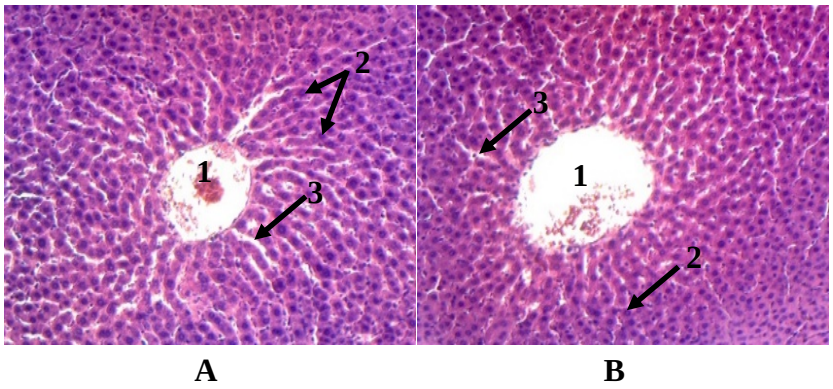


**Figure 1.** Microphotographs of the liver Hematoxylin-Eosin (X 100). A: Normal group; B: Test group; 1: Centrolobular vein; 2: Hepatocyte; 3: Sinusoidal capillary.

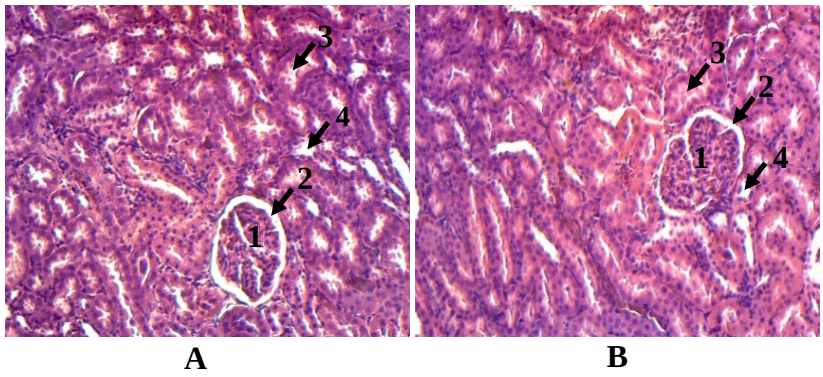


**Figure 2.** Kidney microphotographs Hematoxylin-Eosin (X 100). A: Normal group; B: Test group; 1: Glomerulus; **2**: Urinary space; 3: Proximal bypassed tube; 4: Distal bypassed tube

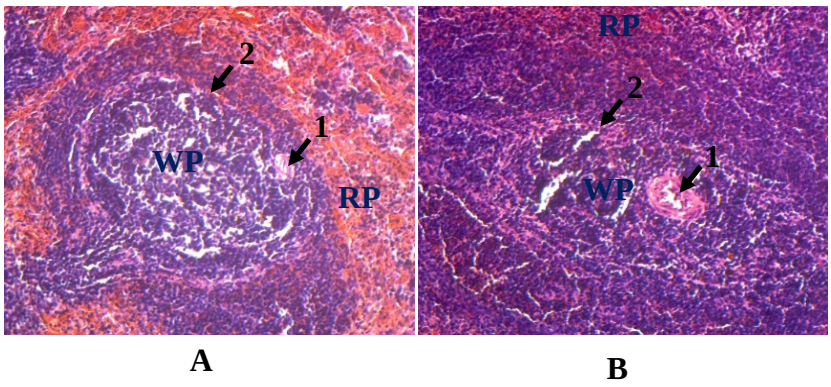


**Figure 3.** Microphotographs of the spleen Hematoxylin-Eosin (X 100). A: Normal group; B: Test group; 1: Arteriolus; 2: Lymphatic nodule; WP: White pulp; RP: Red pulp.

## Hepatic cytolysis and renal functioning

The effects of the extract on the hepatic cytolysis and renal functioning were represented in Table 7. The test group has shown the highest activity of ALAT in comparison to the normal group (100.57 vs 92.10 UI/ml).

**Table 5.** Hematological parameters.

| Parameter | Normal group | Test group |
|---|---|---|
| White blood cells ($10^3$/µl) | 2.72±0.28[a] | 2.60±0.26[a] |
| Red blood cells ($10^3$/µl) | 7.40±1.21[a] | 6.98±0.53[a] |
| Platelets ($10^3$/µL) | 262.50±28.94[a] | 204.75±2.08[b] |
| Granulocytes (%) | 22.85±2.38[a] | 32.40±1.35[b] |
| Lymphocytes (%) | 66.45±2.95[a] | 56.10±5.65[b] |
| Monocytes (%) | 12.40±1.53[a] | 14.50±1.72[a] |
| Hemoglobin (g/dl) | 13.40±2.16[a] | 12.35±1.41[a] |
| Hematocrit (%) | 40.68±7.35[a] | 36.45±3.30[a] |
| MCV ($Um^3$) | 58.95±4.20[a] | 54.38±1.13[a] |
| MPV ($Um^3$) | 7.97±1.06[a] | 8.05±0,93[a] |
| MCH (Pg) | 18.58±0.64[a] | 18.08±0.38[a] |
| MCHC (g/dl) | 31.72±2.50[a] | 33.38±0.90[a] |

The values are expressed as means ± standard deviations. The values assigned different letters on the same line are significantly different at $p<0.05$.

**Table 6.** Parameters of metabolic activities of liver.

| Parameter | Normal group | Test group |
|---|---|---|
| Triglycerides (mg/dL) | 59.25±7.51[a] | 50.31±7.29[b] |
| Total cholesterol (mg/dL) | 83.39±3.59[a] | 66.61±4.64[b] |
| Total proteins (g/L) | 3.76±0.25[a] | 3.43±0.08[a] |

The values are expressed as means ± standard deviations. The values assigned different letters on the same line are significantly different at $p<0.05$.

**Table 7.** Parameters of hepatic cytolysis and renal function.

| Parameter | Normal group | Test group |
|---|---|---|
| ALAT (UI/ml) | 92.10±2.66[a] | 100.57±3.11[b] |
| ASAT (UI/ml) | 60.17±3.34[a] | 66.61±4.64[a] |
| Creatinin (mg/dl) | 1.52±0.15[a] | 1.59±0.20[a] |

The values are expressed as means ± standard deviations. The values assigned different letters on the same line are significantly different at $p<0.05$.

No significant difference was noted with ALAT and creatinin.

## DISCUSSION

*B. toxisperma* is a plant of the Sapotaceae family, therefore potentially toxic because it is made of latex. Therefore, a toxicity study of the hydroethanolic extract of the pulp of the fruit of this plant was carry out. The work began with an acute toxicity study to get an idea of the $LD_{50}$ of our extract. No signs of toxicity were observed for the extract (Table 1) and the $LD_{50}$ was > 2000 mg/kg of body weight. So, this extract could be considered safe and less toxic. Adeneye and Olagunju (2009) already noted that the compounds which have $LD_{50}$ > 1000 mg/kg could be considered safe and low toxic. This limit $LD_{50}$ can justify the choice of the inferior dose for the next part of the study.

Subsequently, a subacute toxicity study was conducted to assess the effects of the extract (400 mg/kg of body weight) following repeated administration in the medium term. In this study, no evidence of systemic toxicity was noted at 400 mg/kg of body weight (Table 2). Since body weight changes are a major indicator of the deleterious effects of toxic substances, including weight loss of 10% from baseline (Wang et al., 2019), the effects of the extract on body weight changes were evaluated and no

weight loss was noted. On the contrary, the evolution was normal compared to the control group (Table 3).

Since the absence of general signs of toxicity was not sufficient to conclude that a substance is safe, the relative weight of the organs firstly assessed. Indeed, the relative weight of the organs provides an idea of whether or not an organ is exposed to a toxic substance (Nirogi et al., 2014). In addition, toxic substances induce abnormal metabolic reactions that can affect organ weights (Tasniya et al., 2017). While the other organs were intact, the extract caused atrophy of the stomach (Table 4). The observed stomach atrophy may be due to possible inflammation or a possible inhibitory effect of epithelial cell proliferation in the stomach (Yo and Nayoung, 2015). To go further, within the organs, histological sections were performed on the liver. These analyses were performed on three of the organs (liver, spleen, and kidneys) most affected by metabolic reactions caused by toxicants (Nirogi et al., 2014). No deleterious effects of the extract on these organs were observed (Figures 1 to 3).

The hematopoietic system is one of the most toxic-sensitive targets and an important index of physiological status (Mukinda and Syce, 2007). Hematological parameters make it possible not only to determine the harmful effects of an extract at the blood level, but also the relationships between the functions of a plant extract or one of its products and blood (Toyin et al., 2007). Hematologically, the extract resulted in an increase in granulocytes and a decrease in platelets (but with a content of 204.75. $10^3$/L in the normal range of 179-373. $10^3$/L) and lymphocytes; the other parameters being unchanged (Table 5). The observed increase in granulocytes would confirm the possible inflammation mentioned with stomach atrophy, with granulocytes being strongly involved and their content increased in case of inflammation. Bafor et al. (2020), had already shown the proinflammatory effects of methanolic extract of *Omphalocarpum procerum* sterm bark. However, the extract could disrupt platelet and lymphocyte synthesis, resulting in low levels observed, which could result in coagulation disorders and decreased specific immunity (Arika et al., 2016). Adebayo et al. (2010) had already shown the antiplatelets property of ethanolic leaves extract of *Chrysophyllum albidum*.

Biochemically, toxic substances can affect enzymatic activities or generate in excess of many metabolites. This as well as ALAT, ASAT are used to explore hepatic cytolysis (Pariente, 2013), triglycerides, and cholesterol for disorders of lipid metabolism in the liver (Warun and Harisha, 2015), as well as total proteins for disorders of protein metabolism. Creatinemia, on the other hand, is a marker of renal dysfunction (Lopez-Giacoman and Madero, 2015). There was an increase in plasma ALAT activity (Table 7) as already observed in the study in diabetic rats. This confirms the capacity of the extract to cause hepatic cytolysis, which would result in impaired liver function and metabolic activity, resulting in decreased total cholesterol and triglyceride levels (Table 6). Indeed, the liver plays an important role in lipid synthesis, and in case of liver damage, its metabolic activity is impaired (Warun and Harisha, 2015).

## Conclusion

The hydroethanolic extract of the fruit pulp of *B. toxisperma* Pierre has an $LD_{50}$>2000 mg/kg of body weight, maintains the morphology and structure of the organs histologically intact, but biochemically is potentially hepatotoxic.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ABBREVIATION

**ALAT;** Alanine Amino Transferase, **ASAT;** Aspartate Amino Transferase, **LD;** Lethal Dose; **MCHC;** Mean Corpuscular Hemoglobin Concentration, **MCH;** Mean Corpuscular Hemoglobin, **MCV;** Mean Corpuscular Volume, **MPV;** Mean Platelets Volume, **NIH:** National Institutes of Health, **OECD;** Organization for Economic Cooperation and Development, **RP;** Red Pulp, **WP;** White Pulp.

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