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RESEARCH ARTICLE

SUBACUTE TOXICITY AND HAEMATOLOGICAL PROFILES OF THE HYDRO-ETHANOL 70 % EXTRACT OF THE TRUNK BARKS OF *TERMINALIA SUPERBA* ENGL. AND DIELS (COMBRETACEAE) IN RATS.

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Abstract

The aim of this study was to assess the safety of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* (HE 70 %) administered orally once daily for 28 days to three groups of albino rats at 250, 500 and 750 mg/kg body weight (b.w.) on haematological parameters and indices. The control group received distilled water while two other satellite groups (control and test group at 750 mg/kg b.w.) were added in order to study the reversibility. Blood was collected into EDTA tubes for haematology at day 0 and at the end of each week. Another 3 ml was collected from the satellite groups, 14 days after the end of the exposure period. These collected blood were used to analyze erythrocytic, leucocytic and thrombocytic profiles. HE 70 % extract does not have an effect on hematocrit, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (leucocytes), lymphocytes, monocytes and granulocytes at all studied doses. On the other hand HE 70 % extract induced a significant increase in red blood cells and haemoglobins at the 4th week at 750 mg/kg b.w. and a significant reduction in mean corpuscular volume (MCV). The blood smear observation did not show any significant anomaly. As for the platelets they knew a significant reduction at all studied doses at the 4th week. This extract would be toxic for the platelets. But this toxicity was reversible.

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Introduction:-

Terminalia superba Engl. and Diels (Combretaceae), is a plant used in treatment of various pathologies. It was subject to many studies in particular those of¹ and² that showed respectively that HE 70 % and the aqueous extract of the trunk barks of *T. superba* possesses anti-ulcerous effect. As for those of³, they showed that the total aqueous extract of the trunk barks of *T. superba* does not have any toxic effect on hepatic and renal functions after 28 days of treatment in rats whereas the long-term use disturbs lipidic metabolism. Moreover⁴, showed that HE 70 % extract of the trunk barks of *T. superba* does not disturb kidneys and liver functioning when it is orally administered to rats during 28 days.

However, plants used as drug have a potential toxicity on blood parameters⁵. It was the case of *Tripterygium wilfordii* Hook (Celastraceae), where⁶ showed that the traditional medicine improven resulting from this plant is

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toxic for the haematopoietic system because it induces leucopenia, thrombopenia and anaemia. Other studies showed that *Catharanthus roseus* (L.) G. Gift (Apocynaceae), plant used in the treatment of diabetes in the Caribbean was strongly haematotoxic and destroyed leucocytes⁷.

In addition, the World Health Organization (WHO), estimated at approximately 80 %, the population of the developing countries which always make recourse to traditional medicine for the care of primary health⁸. This request of traditional medicine is justified partly by impoverishment, insufficiency and lack of hospital structures^{9,10}. This empirical use of traditional medicine exposes the populations to intoxications which prove sometimes tragic¹¹. This is why, although⁴ showed that HE 70 % extract of the trunk barks of *T. superba* is without effect on hepatic and renal functions, and due to the absence of scientific data on the safety of this extract on the haematopoietic system, it is necessary to evaluate its effect on haematologic parameters.

Material and Methods:-

2.1. Material

2.1.1. Collection of plant material

Trunk barks of *T. superba* were collected locally from the forest of Ebilassokro village located at 210 km of Abidjan in the East of Côte d'Ivoire in October 2016. Taxonomical identification of those trunk barks was established by botanist from the National floristic Center of University of Felix Houphouët Boigny, Cocody-Abidjan, Côte d'Ivoire, voucher n°2456, *T. superba* Engl. and Diels in June 4, 1954; n°4207 in March 26, 1957; n°10477, February 26, 1969 and n°416 in April 03, 1974 of Côte d'Ivoire national herbarium.

2.1.2. Extraction process of the hydro-ethanol 70 % extract from the trunk barks of *Terminalia superba*

Extraction is based on *T. superba* traditional method preparation. Trunk barks of *T. superba* were dried under shade and powdered with a machine (mark RETSCH, type SM 100, Germany). The extraction process was implemented according to the method described by¹². One hundred grams (100g) of the trunk barks powder were macerated during 24 hours in 11 ethanol-water (70 :30 v/v) for 3 times until complete exhaustion. The mixtures were filtered (Whatman n°1) and concentrated under reduce pressure using a rotary evaporator (Büchi R110, type MKE 6540/2) at a temperature of 45°C. The concentrated extracts were stored in dessicators (Friocelle®, type D-82166 (Germany)) at 45°C.

2.2. Animals

Albino wistar rats of either sex weighing between 103 and 114 g were selected for this study and were aged from six to eight weeks. They were bred in Animal house of Physiology, Pharmacology and Pharmacopeia laboratory of the University of Nangui Abrogoua (Abidjan, Côte d'Ivoire) according to the principles for the care and use of laboratory animals of the Ethical Committee of the University (Nangui Abrogoua, Abidjan, Côte d'Ivoire). They were exposed to 12 hours dark/light cycle.

2.3. Methods

2.3.1. Subacute toxicity study

It was carried out according to OECD Guidelines 407¹³. Sixty rats were randomly divided in six groups of ten animals including four test group and two control groups. Each group included five male and five female rats. The doses were prepared according to². Doses 250; 500 and 750 mg/kg b.w. corresponding to extract concentrations (12.5; 25 and 37.5mg/ml) were given to groups B, C and D respectively. Group A, served as control group, received distilled water at 2 ml/100 g b.w. Groups B, C and D, received orally HE 70 % extract at 250, 500 and 750 mg/kg respectively. Before the administration of the various treatments, animals of each group were marked and weighed individually. Groups E and F served as satellite groups received distilled water and HE 70 % extract at 750 mg/kg b.w. respectively. These last two groups are included in order to observe reversibility, persistence or late appearance of toxic effects at least 14 days after stopping treatment.

2.3.2. Collection of blood samples

Blood samples from rats in all groups were taken before the commencement of the first oral administration (Day 0) and at the end of each week until the end of the experiment (Day 28) for haematological analysis. Thus, at the day 0 and the end of each week, the fasted animals, the day before evening, were anesthetized with ether and 5 ml of blood was collected early in the morning by ocular puncture in dry centrifuged tubes containing an anticoagulant, ethylene-diaminetetra-acetic acid (EDTA). Fourteen days after stopping the treatment, blood samples of satellite groups were taken according to the same process. A drop of blood from EDTA tubes of each group was used for

blood smear and colouring realization according to¹⁴ method. A binocular optical microscope (Optika, Italy) using the X10 objective with the condenser iris closed sufficiently to give good contrast was used for blood smears observation.

2.3.3. Determination of haematological parameters and indices

Haematological parameters and indices were determined from unclotted blood samples using standard protocols as described by¹⁵. Erythrocytes, haemoglobin concentration, packed cell volume or hematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cells, platelets, white blood cells, neutrophils, monocytes, lymphocytes and granulocytes were determined using an automated haematology analyser Coulter AC Tdiff 2.

2.4. Statistics

The results were reported as mean \pm SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA). The average comparisons was made compared to control, because of ANOVA repeated measurements with mixed model to check variable normality. If the overall P value was found to be statistically significant ($P < 0.05$), further comparisons among groups were made using the Turkey-Kramer multiple post hoc comparison test. All statistical analyses were performed using Graph Pad Prism 6.01 (San Diego, California, USA) software.

Results:-

3.1. Effect of the hydro-ethanol 70 % extract of the trunk barks of *T. superba* on erythrocytes parameters in rats

Oral administration of the hydro-ethanol 70 % extract of the trunk barks of *T. superba* (HE 70 %) to rats induced significant ($P < 0.05$) variations in red blood cell level in the treated groups compared to the control group (figure 1). A highly significant increase ($P < 0.001$) in red blood cell level was observed at 750 mg/kg b.w. compared to the control group in the 28th day of treatment (figure 1). Administration of the extract at the dose levels of 500 and 750 mg/kg b.w caused a significant ($P < 0.05$) increased in haemoglobin level compared to the control group at the 28th day of the treatment (figure 2) while no significant ($P > 0.05$) changes in hematocrit level in the treated groups at all doses compared to the control group was observed for a period of 28 days of HE 70 % extract administration (figure 3). A highly significant ($P < 0.001$) reduction in MCV was recorded at doses of 500 and 750 mg/kg b.w. respectively compared to the control group at the 28th day of extract administration (figure 4). An insignificant ($P > 0.05$) changes in MHC and MCHC levels was observed in the treated groups compared to the control after twenty-eight days of extract administration at the dose levels of 500 and 750 mg/kg b.w (figure 5 and 6). The examination of the blood smears revealed some abnormalities in the size, the form and the colouring in red blood cells. Thus, 90 % of the control group showed normal blood smears (figure 7 and 8) against 80 % in group that received 250 mg/kg b.w. and 70 % in groups that received doses of 500 and 750 mg/kg b.w. (table 1). Some target red blood cells, an hypochromy, a schizocytose, an ovalocyte and target red blood cells associated with a stomatocyte were observed in all the treated animals. All these abnormalities were not significantly ($P > 0.05$) different to those observed in the control group.

3.2. Effect of the hydro-ethanol 70 % extract of the trunk barks of *T. superba* on leucocytic parameters.

As shown in figures 8, 9, 10 and 11, oral administration of HE 70 % extract to rats indicated that no significant ($P > 0.05$) effect in white blood cells, lymphocytes, monocytes and granulocytes levels was observed in the tested group compared to the control group during the 28 days of treatment.

3.3. Effect of the hydro-ethanol 70 % extract of the trunk barks of *T. superba* on platelets

Oral administration of HE 70 % extract, caused a significant decreased in platelet levels ($P < 0.05$) at the doses of 250, 500 and 750 mg/kg compared to the control group in the 28th day of treatment (figure 12).

3.4. Effect of the hydro-ethanol 70 % extract of the trunk barks of *T. superba* on haematological parameters in satellite rat groups.

As shown in table 2, two weeks after the stop of the extract administration, no considerable change in the haematological parameter levels in treated rats were observed compared to the control group. The significant variations observed at the 28th day of the treatment at 750 mg/kg have all disappeared in all the parameters after the interruption of the treatment.

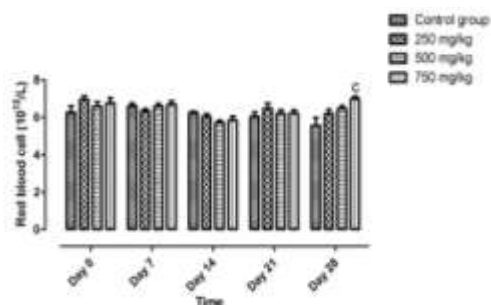


Figure 1:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on Red Blood Cell rate for 28 days in rat. n=10 rats in each group, c: highly significant difference ($P<0.0001$) compared to the control group.

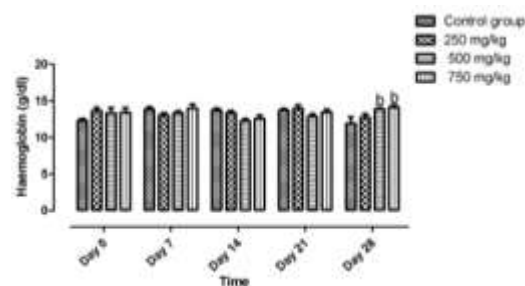


Figure 2:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on Haemoglobin rate for 28 days in rat. n= 10 rats in each group. b: Significant ($P<0.05$). increase compared to the control group

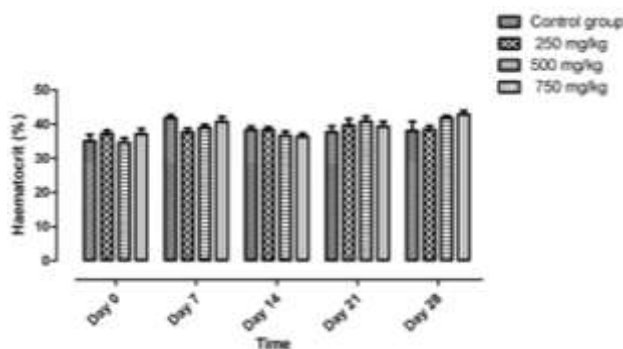


Figure 3:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on Hematocrit rate for 28 days in rats. n= 10 rats in each group. not statistically different from the control group at $P < 0.05$

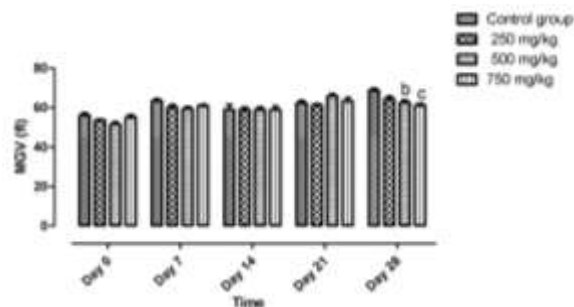


Figure 4:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on MCV rate for 28 days in rat. n= 10 rats in each group. b: $P<0.0001$: Highly significant decrease compared to the control group; c: $P<0.001$: Very significant decrease compared to the control group.

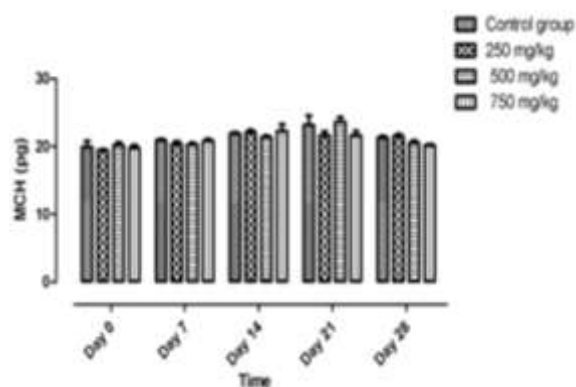


Figure 5:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on MCH rate for 28 days in rats. n= 10 rats in each group. not statistically different from the control group at $P<0.05$

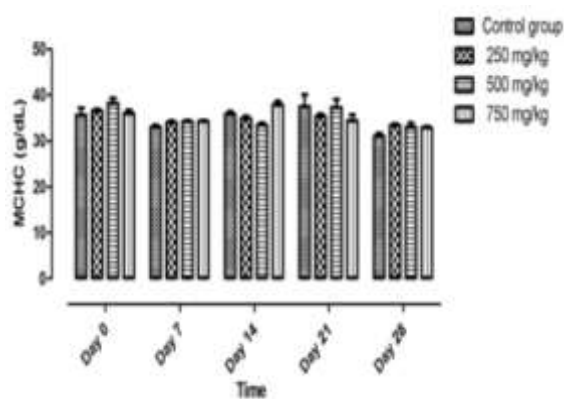


Figure 6:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on MCHC rat for 28 days in rats. n= 10 rats in each group. not statistically different from the control group at $P<0.05$

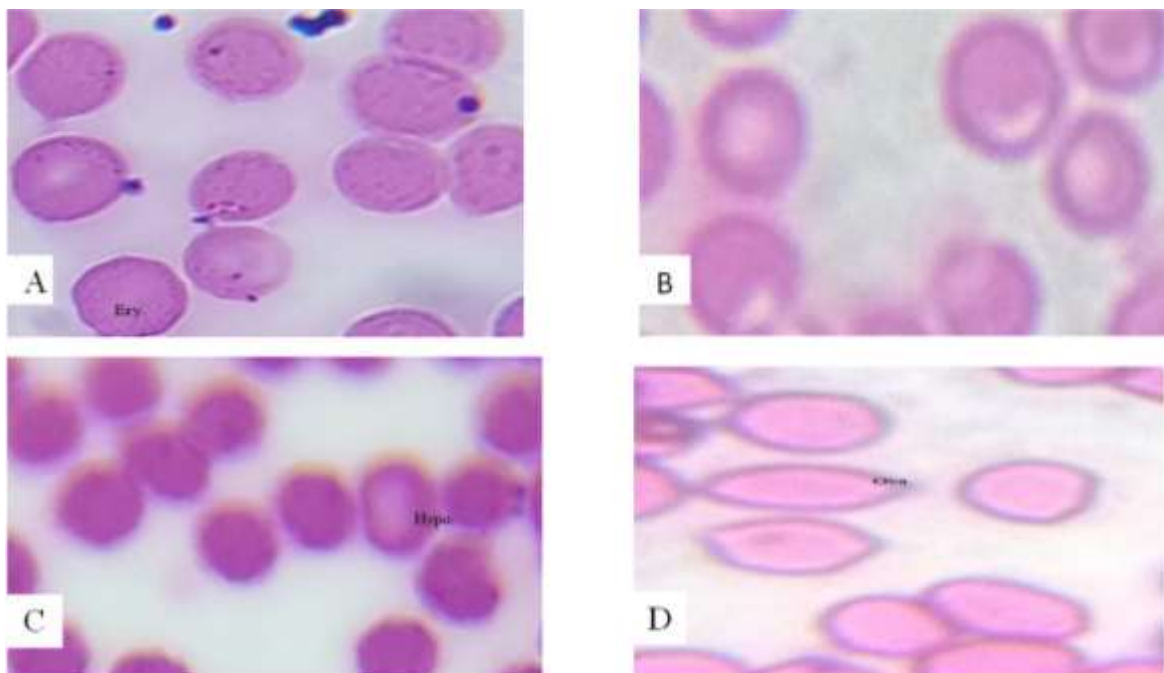


Figure 7:- Blood smears of rats with the hydro-ethanol 70 % extract coloured with May-Grunwald Giemsa, MO, GX100

A: Normal erythrocytes observed in the control group, er:erythrocyte

B: Red blood cells out of targets observed at 250 mg/kg b.w.

C: Hypochromy observed at 500 and 750 mg/kg b.w. and in the control group

D: Ovalocyte observed at 750 mg/kg b.w.

Table 1:- Percentage of the abnormalities observed on the blood smears in rats treated with the hydro-ethanol 70 % extract (colouring May Grunwald Giemsa)

	Doses (mg/kg p.c.)	Normal blood smears	RBCOT	Hypo	Ova.
Subacute toxicity	Control	90 % ns	0 % ns	10 %ns	0 %ns
	250	80 % ns	20 %ns	0 %ns	0 %ns
	500	70 %ns	0%ns	20 %ns	0 %ns
	750	70 %ns	0 %ns	10 %ns	10 %ns

Comparisons are done between the control group and the treated groups of the corresponding study.

n= 10 animals in each group; P>0.05

RBCOT: Red blood cells out of Target; Hypo: hypochromy; Ova: ovalocyte

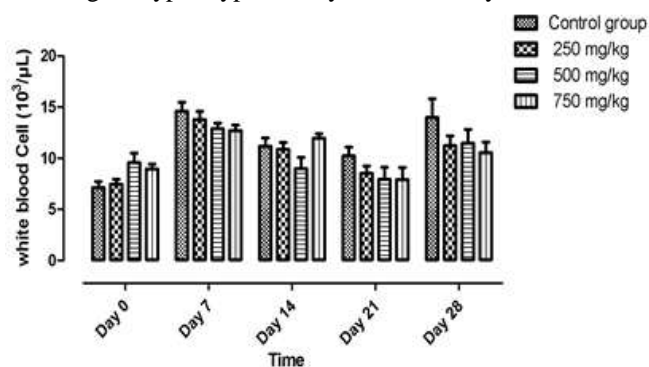


Figure 8:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on white blood cells rate for 28 days. n= 10 rats in each group. not statistically different from the control group at P<0.05

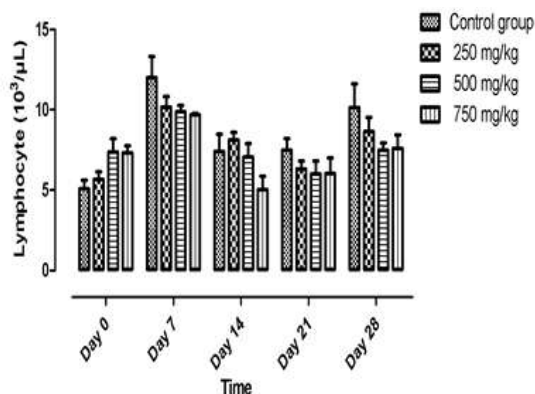


Figure 9:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on lymphocytes rate for 28 days. n= 10 rats in each group. not statistically different from the control group at P <0.05

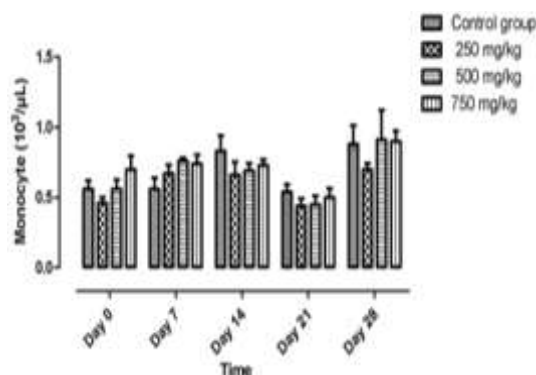


Figure 10:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on monocytes rate for 28 days. n= 10 rats in each group. not statistically different from the control group at P <0.05

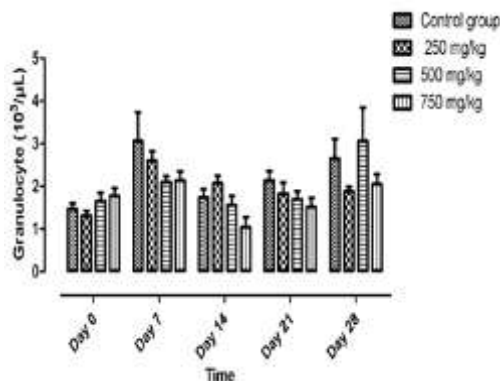


Figure 11:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on granulocytes rate for 28 days. n= 10 rats in each group. not statistically different from the control group at P <0.05

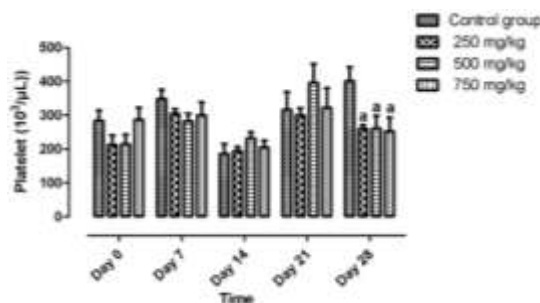


Figure 12:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on Platelets rate for 28 days in rat n= 10 rats in each group. a: P<0.05 significant decrease compared to the control group

Table 2:- Effect of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* on the haematologic parameters of the rats of the satellite groups

Parameters	Control	Day 0	Day 45
Red blood cells ($10^{12}/L$)	Control	6,263 \pm 0,346	8,200 \pm 1,0608
	Test	6,757 \pm 0,284	7,450 \pm 0,5588
Haemoglobin (g/dL)	Control	12,29 \pm 0,257	15,13 \pm 0,3000
	Test	13,42 \pm 0,619	13,82 \pm 1,131
Hematocrit (%)	Control	35,08 \pm 1,885	45,20 \pm 1,026
	Test	37,15 \pm 1,553	40,32 \pm 3,115
MCV (fl)	Control	56,22 \pm 1,148	55,18 \pm 0,6857
	Test	55,18 \pm 1,185	54,23 \pm 1,057
MCH (pg)	Control	19,93 \pm 0,890	18,41 \pm 0,1552
	Test	19,80 \pm 0,437	18,21 \pm 0,3752

MCHC (g/dL)	Control	35,64±1,618	33,47±0,4629
	Test	36,04±0,709	33,75±1,077
White blood cells(10 ³ /μL)	Control	7,120±0,605	12,83±1,029
	Test	8,960±0,471	12,00±1,708
Lymphocytes (10 ³ /μL)	Control	5,090±0,534	10,44±0,8513
	Test	7,330±0,429	9,650±1,429
Granulocytes (10 ³ /μL)	Control	1,470±0,130	1,350±0,1285
	Test	1,770±0,187	1,230±0,1399
Monocytes (10 ³ /μL)	Control	0,560±0,061	1,030±0,08307
	Test	0,700±0,095	1,100±0,2028
Platelets (10 ³ /μL)	Control	284,4±30,57	146,1±18,67
	Test	287,0±35,85	145,6±19,59

n= 10 rats in each group; All the tested groups are compared to the control group ;(P> 0.05).

Discussion:-

A body must relatively keep constant the blood composition and components in normal functioning conditions¹⁶. In this case, haematological studies are important in humans and animals because the blood is the major transport system of the body and both the input and output substances of almost all the body's metabolic processes and any deviations from normal are detectable in the both profile. An evaluation of the haematological profile usually furnishes vital information on the response of the body to injury, deprivation and/or stress. Such an evaluation is indispensably important in arriving at a diagnosis, making a prognosis, assessment of the efficacy of the therapy and toxicity of drugs and chemical substances including plant extracts on the blood¹⁷. It can also be used to explain blood relating functions of chemical compounds or plant extracts¹⁸. In the present study, the oral administration of the hydro-ethanol 70 % extract of the trunk barks of *T. superba* (HE 70 %) induced an increase in the levels of red blood cells and haemoglobin in the treated groups compared to the control group at the 28th day of treatment. A significant decrease in MCV and platelet levels were also observed while no significant variation was recorded during the 28 days of the administration of the extract in hematocrit, MCH, MCHC, white blood cells, lymphocytes, monocytes and granulocytes levels. These increases suggested that this extract contained some chemical compounds able to stimulate the haematopoietin, regulating hormone of the production of the red blood cells on the level of the kidneys. However, reduction in MCV level and the non-variation of MCH and MCHC levels compared to the control group could be explained by the fact that the extract can have a selective effect on the parameters and erythrocytes indices. Indeed, according to¹⁹, a plant extract able to increase erythrocytes parameters and to decrease of it or not to have an effect on others, was selective. These results are similar to those of²⁰ who showed that the leave extracts of *Peristrophe bicalculata* (Retz) are able to increase erythrocytes of experimental animals. On the other hand,²¹ showed that the extract of *Corchorus olitorius* at the doses of 250, 500 and 750 mg/kg did not have any effect on the erythrocytes of experimental rats.

It is well-known that gastric ulcer results from the interruption of the mucous membrane and muscular associated to vascular lesions that entail the losses of blood²². A substance capable to increase the production of the red blood cells and haemoglobin would contribute to treat gastric ulcer. Moreover¹ showed that HE 70 % extract possessed a powerful anti-ulcerous potential. That justifies the use of this extract in traditional medicine in the treatment of gastric ulcer.

The hypochromy and the red blood cells out of target represent a weak colouring of red blood cells i.e. a low rate of haemoglobin²³. However, the quantitative analysis showed a significant rise in haemoglobin. In addition, the qualitative observation (blood smear) did not show any hypochromy and red blood cells out of significant target of the subjects covered compared to rats in control group (explanation). The extract, to the studied doses, would thus not assign the quality and the quantity of the red blood cells. These results are similar to those of²⁴ who showed that the methanolic extract of the leaves of *Vernonia lasiopus* did not significantly assign the quality of the red blood cells at the doses of 50 and 100 mg/kg b.w.

The administration of the hydro-ethanol 70 % extract of the trunk barks of *T. superba* to the rats did not induce any significant modification (p>0.05) on the white blood cells, the lymphocytes, the monocytes and the granulocytes level of the animals in tests groups compared to those of the control group. According to works done by²⁵, the administration of extract of medicinal plant to animals can stimulate or inhibit the factors of regulation of the

proliferation, the differentiation and the maturation of the white blood cells. The non-significant effects on the leucocytes indicate that the extract does not stimulate the immune system with the studied doses and will be well-tolerated by the leucocytic cells. These results corroborate with those of¹. Indeed, these authors showed that the aqueous extract of *Sacoglottis gabonnensis* does not modify the leucocytes. Also these results are different from those of²⁴ who observed that the methanolic extract of *Vernonia lasiopus* increased the rate of white blood cells considerably and this plant contained chemical compounds which stimulate the proliferation, the differentiation and the maturation of the white blood cells. The extract does not stimulate the immune system at studied doses and is well-tolerated by the leucocytic cells. The hydro-ethanol 70 % extract of the trunk barks of *T. superba* caused a significant reduction of platelets compared to the control group. This reduction could be explained by the presence of some compounds in the extract. In addition,¹ indicated the presence of flavonoids in this extract. Works done by²⁷, showed that flavonoids have anti-platelet action by preventing thrombosis. Studies suggested several mechanisms by which flavonoids exert their anti-platelet property by lowering the intracellular level of Ca^{2+} which cause the modification of the metabolism of AMPc and the thromboxane A₂²⁸. Thus, the extract could have a long-term toxic effect on the platelets. After the stop of the administration of the hydro-ethanol 70 % extract of the trunk barks of *T. superba*, no significant modification was observed for all the studied parameters. That supposes that the hydro-ethanol 70 % extract of the trunk barks of *T. superba* does not cause any toxicity effect or that the possible toxic effects observed during the experimentation are reversible.

Conclusion:-

This study showed that the oral administration of the hydro-ethanol 70 % extract of the trunk barks of *Terminalia superba* during 28 days, would be toxic against thrombocytes over a long period of use. However, this toxicity is reversible. It would be thus important that the use of this plant is made with lower dose and with much care.

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Conflict of interest: The authors have declared that no competing interest exists.

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