PHYTOCHEMICAL SORTING AND BIOTOLERANCE OF AN IMPROVED TRADITIONAL MEDICINE USED IN THE TREATMENT OF HEPATITIS B IN CÔTE D'IVOIRE

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ABSTRACT

The use of plants for therapeutic purposes is very old. Even today, they are used by a fraction of the world's population. This work focused on the phytochemical sorting and the evaluation of the acute toxicity of an Ivorian phytomedicine "*ABRAHAM*" used in the treatment of hepatitis B, in Wistar albino rats. To do this, the phytomedicine *ABRAHAM* was administered, in a single dose, orally to four groups of rats, at doses of 500, 1000, 2000 and 5000 mg/kg of body weight. The animals were observed for 14 days. Subsequently, the main chemical compounds as well as hematological and blood biochemical parameters were studied. Phytochemical screening revealed the presence of polyphenols, flavonoids, tannins, quinones, polyterpenes and sterols, alkaloids and saponins. Moreover, the phytomedicine does not significantly disturb the hematological parameters consisting of the rate of red blood cells, white blood cells, blood platelets and hemoglobin. Finally, *ABRAHAM* had no adverse effect on hepatic, renal markers and serum lipid profile of treated rats.

KEYWORDS: Toxicity, *ABRAHAM*, rat, hematological, biochemical.

INTRODUCTION

The plant is a living organism constituting a fundamental link in the biological cycle of other living organisms including the human species (Madi, 2010). It has always been a source of nourishment but can also relieve or even cure certain illnesses. The use of medicinal plants in the treatment of pathologies dates back several millennia. It is now experiencing growth in most countries in the world despite the development of synthetic drugs (Kroa et al., 2014). In all developing countries including Côte d'Ivoire, the use of medicinal plants is the most used means of solving public health problems, especially in rural areas. According to the World Health Organization (WHO), more than 80% of the African population uses traditional medicine for their primary health care due to their proximity and accessibility (WHO, 2002). Traditional medicine benefits from the image of alternative medicine because the substances used, particularly plants, are considered natural treatment products devoid of any risk. However, recent studies have shown that herbal medicine is not always risk-free.

Indeed, several reports worldwide report serious side effects recorded following the use of certain medicinal plants (Kandé et al., 2018). The use of these plants is therefore sometimes the cause of toxicity or interaction problems that can cause therapeutic failures (Owens et al., 2014). These therapeutic failures are due to disruptions in the metabolism of different organs and can even cause death (Najem et al., 2018). In reality, nature is home to a range of toxic plants, including poisonous and hallucinogenic plants, capable of causing serious symptoms (Najem et al., 2018). Safety and harmlessness are therefore important criteria to take into account before administering herbal products. In Ivory Coast, ethnobotanical surveys made it possible to draw up a non-exhaustive list of plant species used in traditional medicine by the populations (Dro et al., 2013). Also, a significant number of works on the biological, pharmacological and phytochemical properties of plants from traditional Ivorian medicine have been carried out. The data provided by these studies made it possible on the one hand to explain the therapeutic action and on the

other hand to confirm the use of these different plants in traditional medicine. However, the area of evaluating the quality and safety of traditional medicines sold on the Ivorian market remains little explored. The scientific valorisation of traditional medicine must lead in particular to the development of plant-based medicines whose safety, effectiveness and quality are certified (WHO, 2004). It is in this context that the present work takes place, which aims to study the biotolerance of a phytomedicine from the Ivorian pharmacopoeia used in the treatment of hepatitis B.

MATERIALS AND METHODS

1.1. Plant material
The plant material is composed of a phytomedicine "Abraham". It is an improved traditional medicine based on medicinal plants.

1.2. Animal material
For this study, rats of the species Rattus norvegicus of the Wistar strain and with an average mass of 92 g were used for the experiment. The rats were provided by the animal facility of the Laboratory of the UFR of Pharmaceutical and Biological Sciences of the Félix Houphouët Boigny University of Abidjan. A total of fifteen (15) rats were randomly distributed into five groups of three (3) animals, including four (4) test groups and a control group. The animals were conditioned at animal room temperature (20-25 °C) with a humidity level of 75 % and a photoperiodicity of 12/24 hours. The diet consisted of IVOGRAIN® granules and the rats were provided with tap water continuously in the feeding bottles. The experimental protocol and animal handling procedures were carried out according to good laboratory practices (OECD, 1998). Figure 2 shows a rat from the animal facility of the Laboratory of the UFR of Pharmaceutical and Biological Sciences of the Félix Houphouët Boigny University of Abidjan.

1.3. Chemical products
Cooper ether for rat anesthesia, a Creatinine/Alkaline picrate kit (BIOLABO), a Cholesterol oxidase/peroxidase kit (BIOLABO) for the determination of cholesterol, a colorimetric enzymatic GPO-POD Triglycerides kit (BIOLABO) for the measurement of triglycerides, a Cholesterol-HDL kit (BIOLABO) direct method for the determination of HDL, an AST/TGO IFCC kit (BIOLABO) for the evaluation of transaminase activity Aspartate aminotransaminase, an ALT/TGP IFCC kit (BIOLABO) for the evaluation of activity transaminases Alanine aminotransaminase. As well as the chemicals used to carry out phytochemical analyses.

1.4. Qualitative phytochemical analysis
Research of sterols and polyterpenes
Sterols and polyterpenes were sought by the Liebermann reaction. Five (5) ml of each of the three extracts were evaporated on a sand bath. The residue is dissolved hot in 1 ml of acetic anhydride; we added 0.5 ml of concentrated sulfuric acid to the triturate. The

appearance, at interphase, of a purple or violet ring, turning blue then green, indicated a positive reaction.

1.4.1. Research of polyphenols
The reaction with ferric chloride ($FeCl_3$) made it possible to characterize the polyphenols. To 2 ml of each extract (etheric, methanolic and aqueous), we added a drop of 2 % alcoholic solution of ferric chloride. The appearance of a more or less dark blue-blackish or green color was a sign of the presence of polyphenols.

1.4.2. Research of flavonoid
Flavonoids were investigated by the cyanidin reaction. Two (2) ml of each extract were evaporated and the residue was taken up in 5 ml of hydrochloric alcohol diluted twice. By adding 2 to 3 magnesium shavings, heat is released followed by a pink-orange or purplish color. The addition of 3 drops of isoamyl alcohol intensified this coloring which confirmed the presence of flavonoids.

1.4.3. Research of tannins
Catechic tannins were investigated using Stiasny's reagent. Five (5) ml of each extract were evaporated to dryness. After adding 15 ml of Stiasny's reagent to the residue, the mixture was maintained in a water bath at 80 °C for 30 min. The observation of a precipitate in large flakes characterized the catechic tannins. For gallic tannins, we filtered the previous solution. The filtrate is collected and saturated with sodium acetate. The addition of 3 drops of FeCl3 would cause the appearance of an intense blue-black color, a sign of the presence of gallic tannins.

1.4.4. Research of free or combined quinones
Quinonic substances were searched for using Bornstraëgen's reagent. Two (2) ml of each of the 3 extracts were evaporated to dryness. The residue is triturated in 5 ml of 1/5 hydrochloric acid. The triturate is poured into a test tube. It is then brought to a water bath for 30 min. After cooling, it is extracted with 20 ml of chloroform. Ammonia diluted 2 times (0.5 ml) was added to the chloroform solution. A red or purple color was a sign of the presence of quinones.

1.4.5. Research of alkaloids
The alkaloids were characterized using Buchard's reagents (iodo-iodide reagent) and Dragendorff's reagents (potassium iodobismuthate reagent). Six (6) ml of each solution were evaporated to dryness. The residue is taken up in 6 ml of 60° alcohol. The addition of 2 drops of Dragendorff's reagent to the alcohol solution caused a precipitate or an orange color. Adding 2 drops of Buchard's reagent to the alcohol solution caused a reddish-brown precipitate and indicated a positive reaction.

1.4.6. Research of saponosides
To look for saponosides, we poured 10 ml of the total aqueous extract into a test tube. The tube was shaken for

15 s then left to rest for 15 min. A persistent foam height greater than 1 cm indicated the presence of saponosides.

1.5. Study of the acute toxicity of the improved traditional medicine

1.5.1. Determination of the concentration of the phytomedicine

To determine the concentration of the medicinal solution, a known volume of the oral suspension of the remedy is transferred into an aluminum or porcelain basin. The bowl is then introduced into an oven for drying for a few days at a temperature of 50°C. The dry extract obtained is weighed on an electronic balance to determine its mass. The mass of the dry extract obtained makes it possible to calculate the concentration of the extract in a suspension can. Thus, for a 500 ml solution contained in the can, we obtained, after drying and weighing, 15.629 mg. The concentration is obtained according to the following formula:

$$C = m/VC = 15629/500 = 31.26 \text{ mg/ml} \quad C = 31.26 \text{ mg/ml}$$

With:
m: the mass of the extract (mg);
C: the concentration of the extract (mg/ml) and
V: the volume of the extract (ml).

1.5.2. Calculation of the volume of solution to administer to rats

It was determined according to the following formula:

$$V \text{ (ml)} = \frac{D \text{ (mg/g)} \times m \text{ (g)}}{C \text{ (mg/ml)}}$$

With:
m: the mass of the subject (mg)
D: the dose used (mg/g body weight);
C: the concentration of the extract (g/ml) and
V: the volume of the extract (ml).

1.5.3. Experimental protocol

The acute toxicity study was conducted in accordance with OECD Guideline No. 423 (2001). The rats were fasted for 24 hours before oral administration of the product. After identification by marking and weighing of the rats, five batches of three rats each were made. These rats were subjected to different treatments. Thus, the animals from batch 1 were treated with distilled water (2 ml/100 g). As for the rats in batches 2, 3, 4 and 5, they were treated with the phytomedicine at respective doses of 500, 1000, 2000 and 5000 mg/kg. Individual observations were made every thirty minutes for the first four hours then every morning between 9 and 10 a.m. over 14 days. These observations included piloerection, aggression, mobility, alertness, stool status, vomiting and mortality. The influence of the different doses administered was assessed based on hematological and blood biochemical data.

1.5.4. Blood sample

Blood samples were taken on the 14th day (D14) of the experiment. In animals anesthetized with ether, blood was collected using a pipette that was slowly introduced into the medial or lateral angle of the eye (Van et al., 1998). For analysis purposes, blood was collected in a dry tube and a tube containing ethylene diamine tetraacetic acid (EDTA) and transported to the laboratory for analysis. The blood contained in the dry tubes was used for biochemical tests and that contained in the tubes containing EDTA were used for hematological analyses.

1.5.5. Hematological analyzes

The blood count was carried out immediately from the samples on EDTA tubes by the URIT-2900® automatic analyzer.

The cell counting principle of the URIT-2900 automaton is based on the variation of impedance. Thus, when a quantity of 10 µl of whole blood is aspirated, the cells pass through a calibrated orifice. A direct current field is applied on either side of this orifice. The cell does not have the same conductivity as the medium surrounding it, its passage through the orifice causes a modification of the current established between the two electrodes. This current difference is recorded, which allows them to be counted each time a cell passes. In addition, the current difference being proportional to the volume of the particle, this is measured at the same time as the counting. Volume is therefore a criterion for differentiating populations of blood cells since platelets and red blood cells on the one hand and leukocytes on the other have distinct volumes most of the time.

In the white blood cell measurement chamber, the sample remains for 10 seconds then the size and number of leukocytes are determined by impedancemetry. In the red blood cell measuring chamber, the size and number of erythrocytes and thrombocytes are also determined by the same method. From the measured values, the other parameters are calculated in the automaton microprocessor. During the incubation time, erythrocytes are dissolved under the influence of lysis and hemoglobin is released and then transformed into methemoglobin. A portion of the sample from this chamber is introduced into the hemoglobin flow trough. The hemoglobin concentration is measured spectrophotometrically.

Before carrying out the count, the blood samples are well homogenized by successive and delicate inversion in order to avoid the formation of microclots. Manually, the sample tubes are presented vertically to the needle of the machine and it is activated on the aspiration button. The sample tube is then withdrawn vertically once the aspiration needle exits the sample. The machine carries out a complete analysis of the hematological parameters

of a sample after one minute to 35 seconds. The URIT-2900 device is connected to a graphics printer which transcribes the results onto paper.

1.5.6. Dosage of biochemical parameters
Blood from the dry tubes was centrifuged at 4000 rpm for 10 minutes. The serum obtained allowed the determination of some biochemical markers of certain vital organs such as the liver and kidneys. Thus, the transaminases which are Alanine aminotransferase (ALT) and Aspartate aminotransferase (ASAT), total cholesterol, triglycerides and HDL cholesterol constituted the hepatic markers. Creatinine was used to assess renal functions.

1.5.6.1. Measurement of alanine aminotransferase activity
The amine group is enzymatically transferred by alanine aminotransferase (ALT) present in the sample from Alanine to the carbon atom of 2-oxoglutarate producing pyruvate and L-glutamate. Pyruvate is reduced to lactate by LDH present in the reagent with simultaneous oxidation of NADH to NAD. The reaction is followed by measuring at 340 nm, the decrease in absorbance due to the oxidation of NADH to NAD+. This decrease is proportional to the activity of Alanine Aminotransferase present in serum by the kinetic method (Gella et al., 1985).
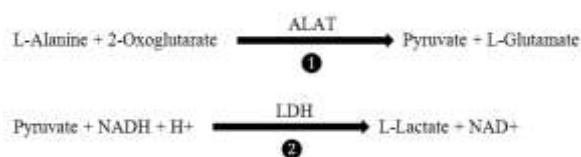

1.5.6.2. Measurement of aspartate aminotransferase activity
The amine group is enzymatically transferred by Aspartate Aminotransferase (ASAT) present in the sample from Aspartate to the carbon atom of 2-oxoglutarate producing oxaloacetate and L-glutamate.


The reaction is followed by measuring at 340 nm, the decrease in absorbance due to the oxidation of NADH to NAD+. This decrease is proportional to the activity of Aspartate Aminotransferase present in serum by the kinetic method (Gella et al., 1985).
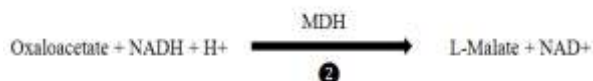

1.5.6.3. Creatinine dosage
The creatinine present in the sample reacts with picric acid in an alkaline medium, to give a colored complex. The rate of formation of this complex is measured in short initial periods, thus avoiding interference from other compounds by the colorimetric method (Bartels & Böhmer, 1971).
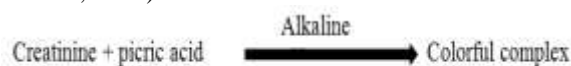

1.5.6.4. Dosage of HDL cholesterol
The direct HDL cholesterol assay is a seamless method for directly measuring HDL cholesterol concentrations in serum or plasma without the need for off-line pretreatment or centrifugation steps. The method is in a two-reagent format and depends on the properties of a single detergent, as shown. This method is based on accelerating the reaction of cholesterol oxidase (CO) with unesterified non-HDL cholesterol and dissolving the HDL selectively using a specific detergent. In the first reagent, unesterified non-HDL cholesterol is subjected to an enzymatic reaction and the generated peroxide is consumed by a peroxidase reaction with DSBmT giving a colorless product. The second reagent consists of a detergent capable of solubilizing HDL specifically, a cholesterol esterase (CE) and a color coupler to develop color for the quantitative determination of HDL cholesterol. This process can be referred to as the selective detergent and accelerator methodology (Barr et al., 1951).
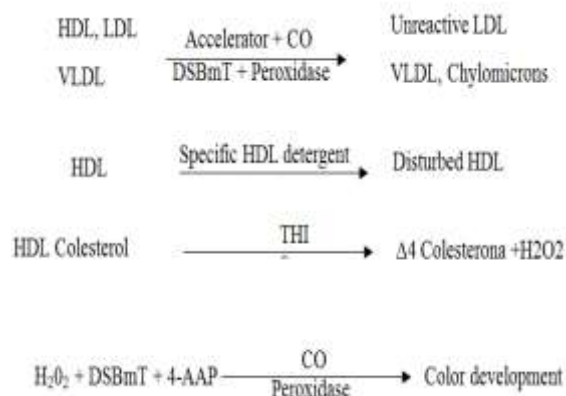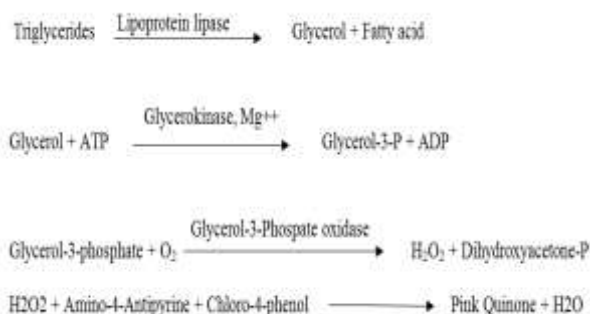

1.5.6.5. Total cholesterol measurement
The total cholesterol (TC) level is quantified according to the method of Fasce et al. (1982). It is measured after enzymatic hydrolysis then oxidation. The quinoneimine indicator is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The enzymatic determination is carried out according to the following reactions:
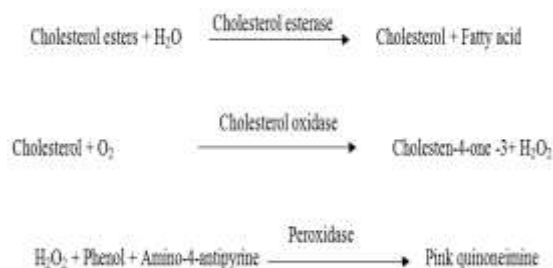
The amount of quinoneimine formed is proportional to the cholesterol concentration. The optical densities are read at a wavelength equal to 505 nm (500-550) after incubation for 5 min at 37 °C. The coloring remains stable for 30 min.

1.5.6.6. Triglyceride dosage
The determination of triglycerides (TG) is done enzymatically. Through the action of specialized lipases, lipoprotein lipase (LPL), triglycerides are hydrolyzed into glycerol and free fatty acid. Glycerol is then converted to glycerol-3-phosphate (G3P) and adenosine diphosphate (ADP) by glycerol kinase and ATP. G3P is then converted by glycerol phosphate dehydrogenase (GPO) to Dihydroxy-acetone (DAP) and hydrogen peroxide (H_2O_2). The latter reacts with 4-aminophenazone (4-AP) and p-chlorophenol in the presence of peroxidase (POD) giving a compound colored red. The reaction scheme is as follows:

Triglycerides — Lipoprotein lipase → Glycerol + Fatty acid

Glycerol + ATP — Glycerokinase, Mg++ → Glycerol-3-P + ADP

Glycerol-3-phosphate + O_2 — Glycerol-3-Phospate oxidase → H_2O_2 + Dihydroxyacetone-P

H2O2 + Amino-4-Antipyrine + Chloro-4-phenol ———→ Pink Quinone + H2O

The concentration of colored quinone (pink) measured at 505 nm (500-550) is directly proportional to the quantity of TG contained in the sample (Fossati et al., 1982).

1.6. Statistical analysis
The statistical study was carried out using the XLSTAT-PRO 7.1 statistical analysis computer software. Results were analyzed using Dunnett's post hoc tests combined with one-way ANOVA. Values are given as means followed by the standard error of the mean. The different statistical tests are considered significant when $p < 0.05$ and very significant when $p < 0.01$.

RESULTS
3.1. Phytochemical sorting
The phytochemical test made it possible to highlight the presence of families of chemical compounds in our extract. We noted the presence of alkaloids, flavonoids, polyphenols, quinones, saponosides, sterols and tannins. The results of this study are recorded in Table I.

Table I: Some qualitative constituents of the improved traditional medicine.

Samples	Sterols (Polyter-bolts)	Polyphenols	Flavo-noids	Tannins		Quinones	Alkaloids		Saponosides
				cat	gal		Bou	Dra	
Reactions	+	++	++	++	-	+	+	+	++

cat: catechisms gal: gallic Bou: Bouchardat Dra: Dragendorff
-: Absence of the desired substance
+: Low intensity presence of the desired substance
++: Presence of the desired substance at medium intensity

3.2. Effect of improved traditional medicine on hematological parameters
3.2.1. Effect of phytomedicine on white blood cells
The evolution of the number of white blood cells after administration of the different doses of the improved traditional drug indicates a slight increase in the white blood cells in the rats of batches 2, 3 and 5 compared to the number of white blood cells of the animals in the control batch. However, we observed a greater increase (12,600 cells/µl) in the rats from batch 4 compared to the number of white blood cells in the animals from the control batch (8,917 cells/µl) as shown in Figure 3.
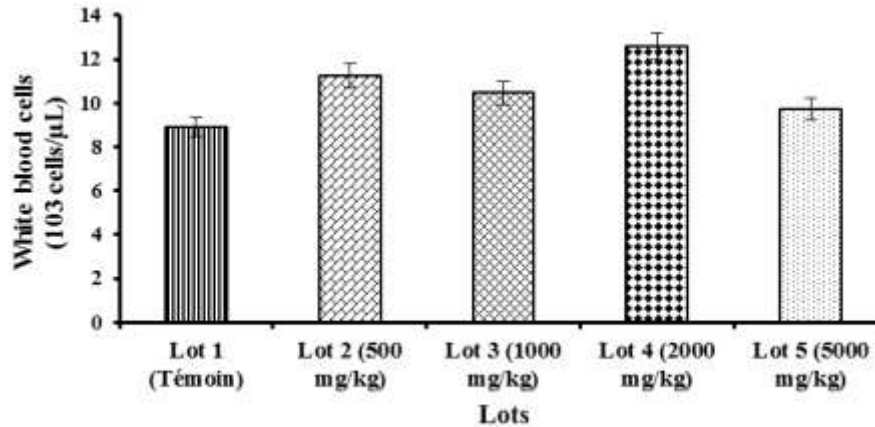
Figure 3: Evolution of white blood cells after treatments with the phytomedicine.

3.2.2. Effect of phytomedicine on red blood cells
Administration of the phytomedicine resulted in a significant increase ($p<0.05$) in the number of red blood cells in rats from batches 2, 3, and 4 compared to that of red blood cells in rats from the control batch. On the other hand, there is no significant difference between the number of red blood cells in the animals of batch 5 (5.637 cells/µl) and that of red blood cells in control rats (5.037 cells/µl). These results are presented in Figure 4.
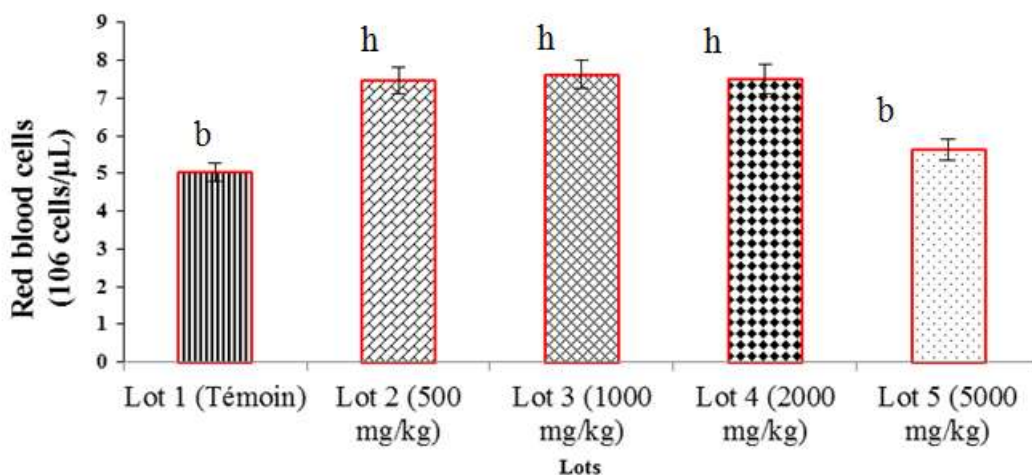

Figure 4: Evolution of red blood cells after treatments with the phytomedicine.

3.2.3. Effect of phytomedicine on hemoglobin level
The hemoglobin level of rats from batches 2, 3 and 4 significantly increased ($p<0.05$) compared to that of rats from the control batch (Figure 5). However, the hemoglobin level of rats in batch 5 remained similar to that of control rats.
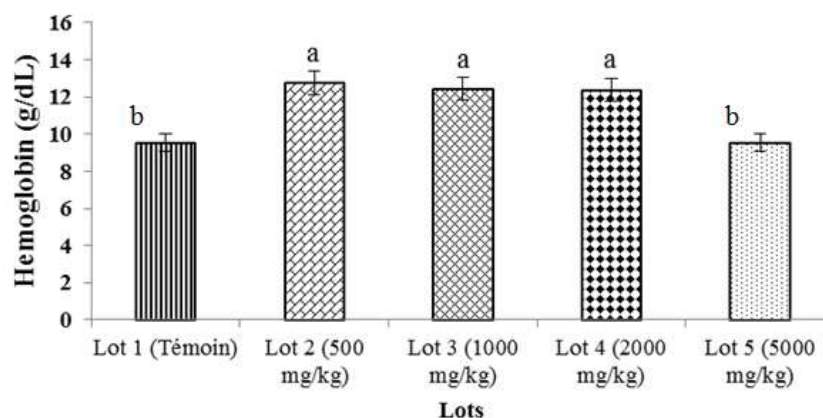

Figure 5: Evolution of hemoglobins after treatments with the phytomedicine.

3.2.4. Effect of phytomedicine on blood platelets

The administration of the phytomedicine led to a significant increase ($p<0.05$) in the number of blood platelets in rats from batches 2, 3, and 4 compared to the number of the same blood constituent in rats from the control batch. However, we do not notice any significant difference between the number of blood platelets (450 cells/µl of blood) in the rats from batch 5 compared to that of the rats from the control batch (500 cells/µl of blood) as presented in the figure. 6.
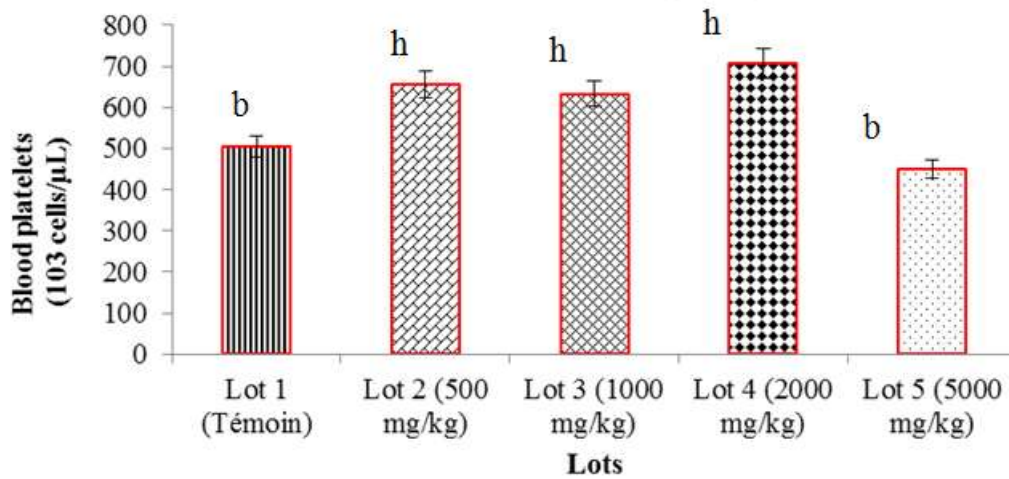

Figure 6: Evolution of blood platelets after phytomedicine treatments.

3.3. Effect of improved traditional medicine on biochemical parameters

3.3.1. Effect of phytomedicine on transaminases

The average values of transaminase (TGO or ASAT) of the batches treated with the phytomedicine suffered a significant decrease ($p<0.05$) compared to those of the rats in the control batch (Figure 7). At the level of transaminase (TGP or ALT), the average values of the batches treated with the phytomedicine experienced a significant ($p<0.05$) and very significant ($p<0.01$) increase compared to those of the control batch (Figure 8).
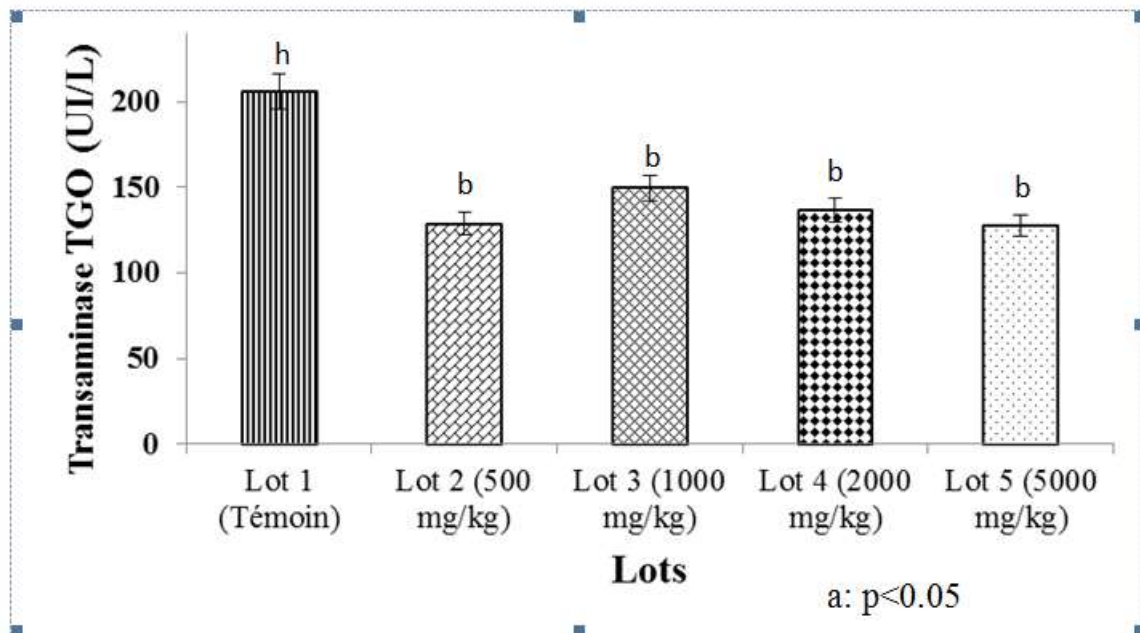

Figure 7: Effect of phytomedicine on TGO activity in rats.

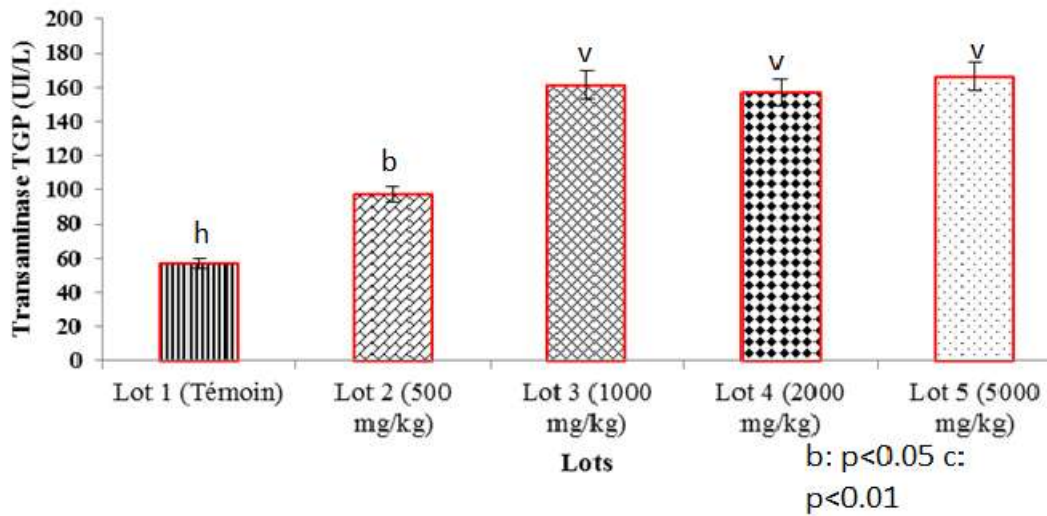**Effect of the phytomedicine on transaminases (TGP or ALT)**

Figure 8: Effect of phytomedicine on TGP activity in rats

3.3.2. Effect of phytomedicine on creatinine
The average creatinine value of rats from batch 2 experienced a slight increase (7,100 μl) compared to that of controls (5.833 mg/dl) while that of rats from batches 3, 4 and 5 suffered a slight decrease (Figure 9).
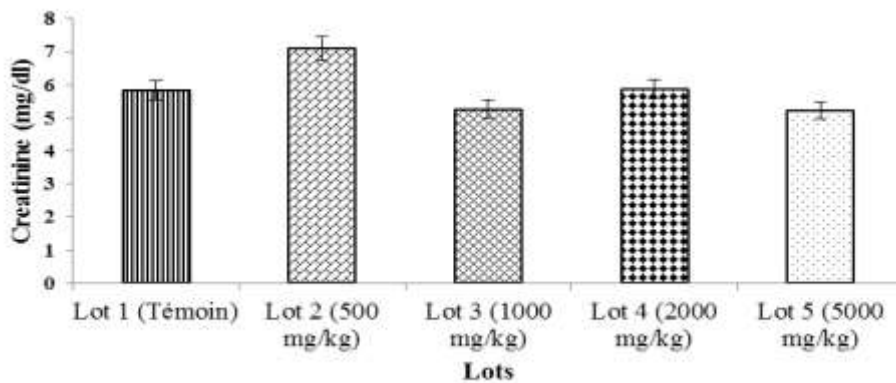

Figure 9: Effect of phytomedicine on creatinine.

3.3.3. Effect of phytomedicine on total cholesterol
The cholesterol concentration of rats in batches 2, 3 and 5 increased compared to that of controls after administration of the phytomedicine. However, we notice a slight decrease in this rate in the rats from batch 4 (0.730 g/l) compared to that of the rats from the control batch (0.747 g/l) as appears in Figure 10. Administration of the phytomedicine did not significantly disturb the serum total cholesterol level in the treated groups compared to the level observed in the controls.
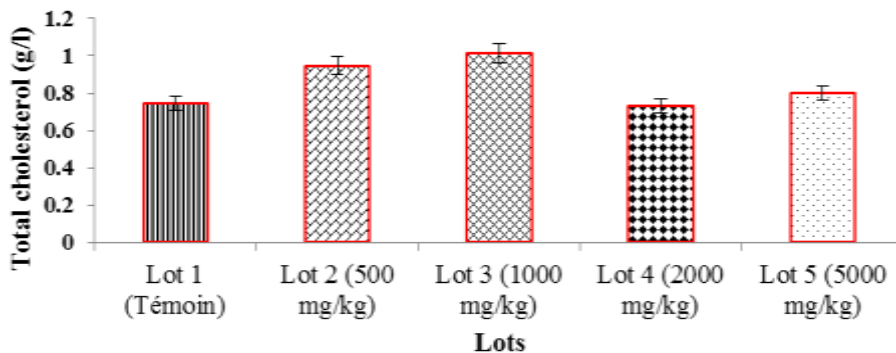

Figure 10: Effect of phytomedicine on total cholesterol.

3.3.4. Effect of phytomedicine on triglycerides

A drop in average triglyceride values was observed in all the batches treated with the phytomedicine compared to the control batch (Figure 11).
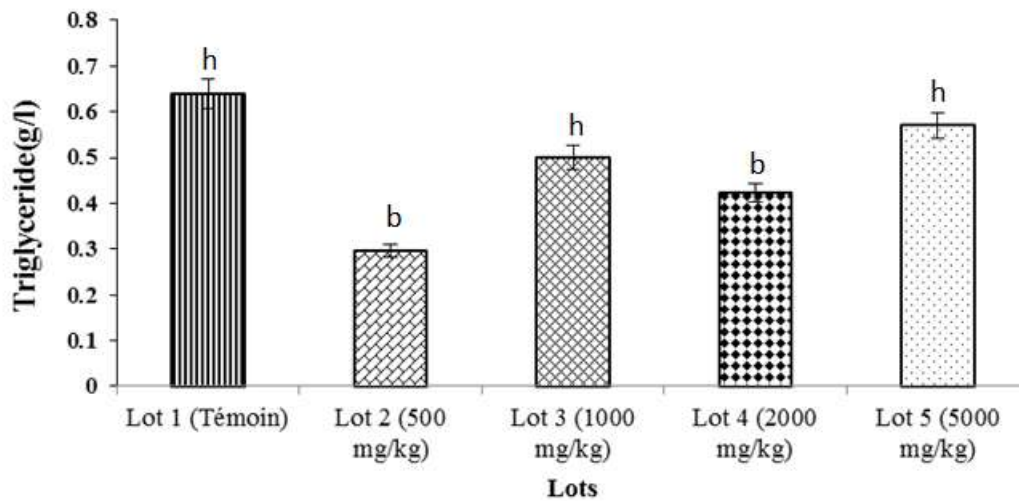

Figure 11: Effect of phytomedicine on triglycerides

3.3.5. Effect of phytomedicine on HDL cholesterol

The average concentration of HDL cholesterol increases significantly($p<0.05$)in all rats from batch 2 (0.353 g/l) and batch 5 (0.320 g/l) treated with the phytomedicine at doses of 500 mg/kg body weight and 1000 mg/kg body weight respectively, compared to that of control rats (0.240 g/l), in accordance with Figure 12. On the other hand, no significant difference was observed between the average concentrations of batches 3 and 4 treated with the phytomedicine respectively at doses of 1000 mg /kg of body weight and 2000 mg/kg of body weight and those of the control batches.
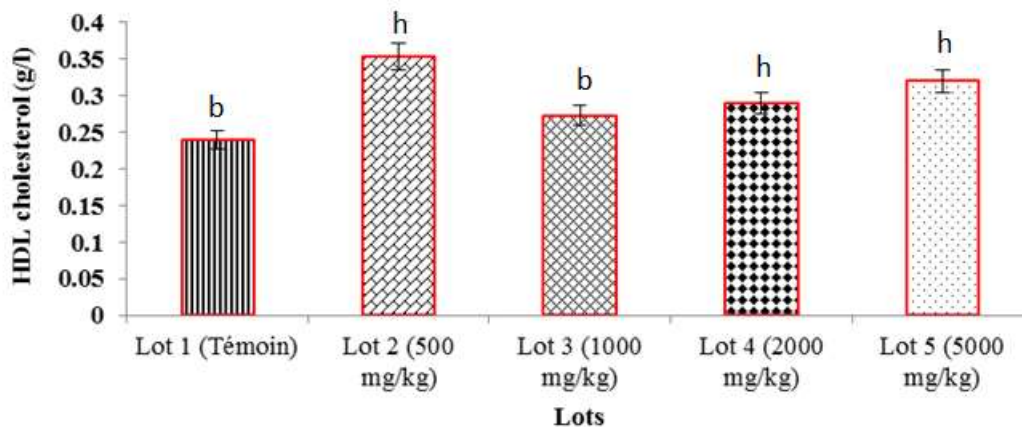

Figure 12: Effect of phytomedicine on HDL cholesterol.

4. DISCUSSION

The results of the qualitative phytochemical study revealed that the phytomedicine contains several chemical groups, namely: alkaloids, flavonoids, polyphenols, polyterpenes, quinones, saponins, sterols and tannins. These results are in agreement with those of Akakpo et al. (2020), which in addition to these compounds, highlighted other compounds such as reducing compounds and protoanthocyanins which were not looked for in this study.

The richness of this phytomedicine in active chemical compounds could explain the traditional use of the plants which compose it to treat numerous diseases such as amenorrhea, headaches, diabetes, fever, migraine, edema, rheumatism and 'gastric ulcer. Several studies have shown that these secondary metabolites are important indicators for certain pharmacological activities. Indeed, tannins are phenolic compounds known for their antioxidant properties (Ebrahimzadeh et al., 2010). These antioxidants could thus activate the immune defense and reduce the risks of cancer and

degenerative diseases (Mpondo et al., 2012). As for saponins, they have surfactant, antifungal, antibacterial and antiviral properties. They would also present protective activities of veins and capillaries and edematous activity with hormonal activity (Macheix et al., 2005). Alkaloids are believed to have several biological properties (Okwu, 2007). According to Badiaga (2011) alkaloids are highly sought after for their broad spectrum of biological activities including antibiotic, antiparasitic, anesthetic, antitumor, anticancer and analgesic and spasmolytic properties. Alkaloids are thought to have actions on the central nervous system (Bruneton, 1999). Sterols and polyterpenes are used for their antipyretic and analgesic properties. They regulate the metabolism of proteins and carbohydrates and increase the synthesis of muscles and bones (Hossain et al., 2013). Flavonoids are endowed with antioxidant, anti-inflammatory (Wang and Mazza, 2002) and analgesic (Bittar et al., 2000) properties. Studies have also shown that certain flavonoid compounds possess sedative, anticonvulsant, and immunomodulatory properties (Lyu and Park, 2005).

The results of the acute toxicity study indicated an absence of signs of toxicity after administration of doses ranging from 500 to 5000 mg/kg body weight (bw) of the phytomedicine "ABRAHAM". All animals survived after 14 days of observation, which implies that the LD50 of the phytomedicine is greater than 5000 mg/kg bw. According to OECD Guideline 425 for Testing of Chemicals, the extract is classified as GHS Category 5 and considered a non-toxic substance orally (OECD, 2008). This result is in harmony with that of Ayoola et al. (2020) who after administration of the ethanolic extract of the leaves of M. acuminata at a dose of 5000 mg/kg bw observed no clinical signs and no deaths in rats after 14 days of observation. Researchers have also shown that the LD50 of the aqueous extract of Cymbopogon citratus (Poaceae) is greater than 5000 mg/kg bw after a single administration in rats (Adeneye et al., 2007).

The complete blood count was used in this study to quantify the level of white blood cells, red blood cells, hemoglobins and blood platelets in the blood of rats. During the present study, we observed an increase in the number of white blood cells, red blood cells, blood platelets and the hemoglobin level of the batches treated with the phytomedicine compared to the control batch. An increase in the number of red blood cells and hemoglobin levels in rats treated with ABRAHAM would be beneficial in preventing anemia often associated with the presence of plasmodium in patients. These results corroborate those of Sanogo et al. (2007) who showed that the administration of phytomedicine based on Argemone mexicana at doses of 300 mg/kg and 1500 mg/kg leads to an increase in the hemoglobin level and the number of red blood cells. The increase in white blood cell and platelet levels in treated rats directly indicates a strengthening of the immune system (Hariri et

al., 2011). This suggests that ABRAHAM contains bioactive substances which have an amplifying power of the immune response by increasing the level of white blood cells: the first defensive level of the body (Atsamo et al., 2011). Studies by (Kumar et al., 2011) have shown that flavonoids and tannins participate in strengthening the immune system. Their results are therefore in line with ours. Likewise, Metayer et al. (2015) in a study showed that consumption of foods with a high content of alkaloids (5050 µg/kg of food) leads to a significant increase in the concentration of white blood cells, lymphocytes and blood platelets, thus reflecting a stimulation of the immune defense. The alkaloids in our extract could be the basis of our results.

The results showed that the administration of the phytomedicine Abraham to the animals led to a decrease in aspartate aminotransferase compared to the controls, unlike alanine aminotransferase which increased at all doses. Indeed, AST and ALT are enzymes of mitochondrial and cytoplasmic origin commonly analyzed to assess liver damage (Dillon and Miller, 2016). Any cell necrosis, destruction of the liver parenchyma or an increase in the membrane permeability of hepatocytes leads to the flow of these enzymes into the blood circulation and therefore to an increase in their serum levels (Manda et al., 2017). Their activity is proportional to the degree of liver damage and are therefore good indicators of hepatotoxicity (Pariente, 2013). According to studies by Senior (2012), cytosolic alanine aminotransferase is, in concentration, much higher in the liver than aspartate aminotransferase. The increase in ALT levels is therefore not harmful to the liver in the context of our study. The decrease in the AST level shows that the liver and to a lesser extent the muscles have not been affected. Furthermore, the chemical composition of ABRAHAM could provide serious clues that could enable the identification of the chemical compounds responsible for its effect on liver enzymes. Indeed, according to our study, the phytomedicine is rich in flavonoids, molecules known to be hepatoprotective (Narayana et al., 2001). The effect of the phytomedicine on the liver is therefore due to the presence of flavonoids. These results mean that ABRAHAM had no deleterious effects on the liver function of rats. Creatinine is an excellent marker of renal function, its increase or decrease reflects renal dysfunction (Sirwal et al., 2004). The results of our work did not indicate any disturbance in terms of creatine levels. This indicates normal kidney function. These results are different from those of Gbogbo et al. (2014) who showed the toxic effect of a phytomedicine on renal function by increasing the serum creatinine level. The lipid profile carried out using the determination of total cholesterol, HDL cholesterol and triglycerides indicated no statistically significant difference between the batches of treated rats and those of control rats except the triglycerides of rats from all batches, and at all times. doses, which suffered a significant drop compared to those of the control batches. Similar results were

obtained in other work carried out on phytomedicines in Wistar rats (Adebayo et al., 2010). In short, ABRAHAM did not cause any disturbance in lipid parameters when administered at doses of 500 mg/kg, 1000, 2000 and 5000 mg/kg body weight.

CONCLUSION

The present work focused on the phytochemical sorting and evaluation of the acute toxicity of an Ivorian phytomedicine "ABRAHAM" used in the treatment of hepatitis B. During this work, phytochemical studies based on specific tests were carried out. showed the presence of polyphenols, flavonoids, saponins, alkaloids, quinones, polyterpenes and sterols in the phytomedicine. In addition, the extract administered orally does not present acute toxicity for a dose less than or equal to 5000 mg/kg. No signs of behavioral toxicity were observed. Furthermore, ABRAHAM had no adverse effects on hematological parameters as well as liver biochemical parameters and lipid profile. Considering the results obtained, we can deduce that the product did not present acute toxicity for the majority of the parameters tested, and therefore had no influence on blood quality and function. This practically shows the safety of the phytomedicine ABRAHAM, a traditional recipe used in the treatment of hepatitis B in Ivory Coast. All these results suggest the importance and growing place of the use of medicinal plants in the treatment of certain pathologies in Africa, in general, and in Côte d'Ivoire, in particular. However, other work such as the action of ABRAHAM on blood sugar and chronic oral toxicity tests deserve to be carried out in order to confirm its non-toxic nature.

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