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Comparative effect of extracts of *Blighia sapida* (Sapindaceae) from three regions on the biochemical parameters of hypertensive rats

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Abstract

Blighia sapida is a plant with many therapeutic virtues. In Ivory Coast, this plant is widespread in several regions with different climatic conditions. Our study aims to compare the effects of aqueous extracts of *Blighia sapida* harvested in three Ivorian regions on the biochemical parameters of rats made hypertensive by a fructose diet. Rats (20) were fed a high fructose diet (70% fructose, 10% fat, 20% protein) for 30 days. Then blood pressure was measured from an armband to the tail of the rats. Finally, biochemical and lipid parameters were measured before and after the fructose-enriched diet. All rats fed the high-fructose diet had similar rates of hypertension (SBP = 160.0 ± 0.07 mmHg; DBP = 141.5 ± 1.21 mmHg; HR = 325.7 ± 1.52 beats/min) and a change in dosing parameters. Aqueous extracts of *Blighia sapida* from Adzopé (EAA) and Korhogo (EAK) administered to hypertensive rats normalized the previously increased cardiovascular, biochemical, and lipid parameters compared to control rats (healthy and untreated diseased rats). However, the aqueous extract of *Blighia sapida* of Adzopé (EAA) showed the best effects on hypertension compared to the aqueous extract of *Blighia sapida* of Korhogo (EAK). Indeed, the more humid the region and the climate, the more effective the extract is. The regulating effect of aqueous extracts of *Blighia sapida* on blood pressure, therefore, differs from one region to another with different climatic conditions. This would explain its use in traditional medicine in certain climatic zones compared to others where its therapeutic effects would be unknown because less. Adzopé would, therefore, be the best region where *Blighia sapida* should be harvested for the optimal treatment of high blood pressure.

Keywords: *Blighia sapida*, biochemical parameters, hypertension, Sapindaceae.

1. Introduction

The use of plants for therapeutic purposes has been known by our ancestors and parents for a very long time (**Mamyrbekova-Békro et al., 2011**). In recent decades, 70-95% of the world's population has been using medicinal plants for primary care due to a lack of access to the drugs prescribed by modern medicine but also because plants have been able to demonstrate real efficacy (**Selles, 2012**). Thus, the African pharmacopeia, which aims to develop recipes or formulas that only traditional therapists have the secret, deserves to be highlighted by scientific support given the increasingly considerable fringe of the population that uses it.

To this end, several investigations have been carried out to provide a scientific approach to the use of this traditional medicine. This led to the discovery of a large number of medicinal plants, including *Blighia sapida*. *Blighia sapida* is a medium-sized tree of the Sapindaceae family native to West Africa and cultivated in tropical regions for its edible fruit. In Nigeria, the bark, stem, and leaves of *Blighia sapida* are used in the treatment of fever, malaria, hemorrhage, dysentery, yellow fever, diabetes, constipation, prevention of degenerative diseases, and reduction of cancer and cardiovascular disease (**Okwu et al., 2006; Dossou et al., 2014**). In Ghana, root bark is a traditional antibiotic used mainly for the treatment of diarrhea (**Antwi et al., 2009**). In Côte d'Ivoire, *Blighia sapida* is widespread in several regions with different climatic conditions and its bark is used to treat high blood pressure.

The objective of this work is to compare the effect of aqueous extracts of *Blighia sapida* from three Ivorian regions on the biochemical parameters of rats made hypertensive by fructose.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

The plant material consists of *Blighia sapida* bark. The bark was harvested in the forest region of Adzopé (South-East) and in the savannah region in Korhogo (North).

2.1.2. Animal material

Rats of the species *Rattus norvegicus* strain Wistar were used for this study. They were provided by the Animal Physiology Laboratory of the Biosciences UFR of the Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). The animals kept in plastic cages with stainless steel covers were acclimatized in the animal house of the Ecole Normale Supérieure (ENS) (Abidjan, Côte d'Ivoire). The cages contained a litter of wood shavings renewed every two days throughout the experiment. The experiments were carried out according to a good practice guide to the administration of substances and removal of blood, including routes and volumes with rules and guidelines established for the care of laboratory animals (**Diehl et al., 2001**).

2.2. Methods

2.2.1. Preparation of extracts

The bark of *Blighia sapida* harvested was washed and cut into small pieces. They were dried in the shade of the sun at room temperature. The dried bark was made into a fine powder and the aqueous extract was obtained according to the method used by **Talbi et al. (2014)**. According to this method, 300g of *Blighia sapida* powder was mixed with 3L of distilled water. The aqueous mixture was stirred for 48h at 80°C using a magnetic stirrer type IKA-MAG RCT. The homogenate obtained was filtered twice successively on cotton wool and then on Büchner with Wattman paper. The filtrate was evaporated under reduced pressure at a temperature of 50 °C using a BÜCHI rotary evaporator. The dry evaporate was recovered in powder form, thus constituting the total aqueous extract used for any further work.

2.2.2. The qualitative and quantitative study of secondary metabolites

Phytochemical screening was carried out according to the methods used by **Bidié et al. (2011)** and **Coulibaly et al. (2017)**. These studies were based on staining tests, precipitation tests, and ultraviolet-light observations.

Quantification of the content of secondary metabolites in *Blighia sapida* extracts was carried out using spectrophotometric methods applied by **Fadili et al. (2017)** and **Evenamede et al. (2017)**. Thus, the total polyphenol content of the extracts was determined by the Folin-Ciocalteu method and the aluminium trichloride $AlCl_3$ method was adopted for the determination of total flavonoids.

2.2.3. Induction of hypertension

The rats (24) were divided into two lots, lot G1 of four rats and a second lot G2 of twenty rats. Lot (G1) representing the control group was fed pellets and distilled water, and test lot G2 received the fructose diet for 30 days. During this period, cardiovascular and biochemical parameters were measured every five (05) days until the end of induction. At the end of the 30 days, the animals were left untreated and observed for five (05) days and the cardiovascular and biochemical parameters were measured again.

2.2.4. Treatment of animals rendered hypertensive

After the determination of hypertension, the animals in test batch G2 were subdivided into batches of four rats each (G2' to G6) and treated for seven (07) as follows :

G1: control rats;

G2' sick control rats;

G3: untreated diseased control rats;

G4: rats treated with Adzopé aqueous extract (EAA) at a dose of 400 mg/Kg. bw per day;

G5: rats treated with Korhogo aqueous extract (EAK) at 400 mg/Kg. bw per day;

G6: rats treated with nifedipine (NIFE) at a dose of 10 mg/Kg. bw per day.

At the end of treatment, biochemical parameters such as transaminases (ASAT and ALAT), urea, creatinine, lactate dehydrogenase, and lipid profile were determined using a Cobas Integras automaton.

Statistical analysis

The recorded data were processed using GraphPad software version 7. For each variable, the mean (M) and the standard deviation of the mean (SD) were calculated. The results of the different groups were compared using the analysis of variance (ANOVA) of Turkey and Dunnett. The level of statistical significance of the results was set at $p < 0.05$.

3. Results

3.1. Result of the spectrophotometric determination of total polyphenols and flavonoids

The determination of total polyphenols in aqueous extracts of *Blighia sapida* gave the results presented in **Table I**. These results show that the highest content (13.77 ± 0.76 mg EAG/g) was found with the aqueous extract from the Adzopé region followed by the aqueous extract from the Korhogo region (11.11 ± 1.01 mg EAG/g). EAA also recorded the highest total flavonoid content (3.76 ± 0.00 mg EQ/g) followed by EAK (3.38 ± 0.04 mg EQ/g). The concentration of total polyphenols and flavonoids, therefore, differed from region to region.

Table I: Result of the determination of total polyphenols and flavonoids of aqueous extracts

Extracts	EAA	EAK
Polyphenols (mg EAG/g)	13.77 ± 0.76	11.11 ± 1.01
Flavonoids (mg EQ/g)	3.76 ± 0.15	3.38 ± 0.04

EAA: Adzopé Aqueous Extract; EAK: Korhogo Aqueous Extract

3.2. Effect of fructose on cardiovascular parameters

Ingestion of the fructose diet for 30 days by G2 rats caused a very significant increase in cardiovascular parameters. During this permanent increase in blood pressure (SBP from 122.2 ± 1.27 mmHg to 160.0 ± 0.07 mmHg; DBP from 102.7 ± 3.11 mmHg to 141.5 ± 1.21 mmHg and cardiac pool (HR from 239.7 ± 1.15 beats/min to 325.7 ± 1.52 beats/min), the results of the assay of certain biochemical parameters showed a very significant increase in urea, creatinine, transaminases (AST and ALT) and LDH in rats fed the fructose diet.

3.3. Effect of aqueous extracts of *Blighia sapida* on serum markers of kidney, heart, and liver during treatment

Figures 1; 2 and 3 show the results of the pharmacological effect of aqueous extracts of *Blighia sapida* on serum markers of the kidney,

heart, and liver in fructose-hypertensive rats. These figures show that excessive ingestion of the fructose diet significantly increased serum levels of urea (A) (from 0.44 ± 0.03 mmol/L to 2.11 ± 0.11 mmol/L), creatinine (B) (from 23.08 ± 1.97 μ mol/L to 63.66 ± 3.60 μ mol/L), ASAT (C) (156.2 ± 1.04 U/L to 200.0 ± 0.04 U/L), ALAT (D) (72.2 ± 3.01 U/L to 127.5 ± 3.34 U/L) and LDH (E) (184.5 ± 4.45 IU to 275.7 ± 5.45 IU). Treatment of hypertensive rats with 400 mg/kg bw of various aqueous extracts of *Blighia sapida* significantly decreased the increase in serum levels of these serum markers of the kidney, heart, and, liver caused by the fructose diet. The aqueous extract from the Adzopé region showed the best effects on biochemical disorders caused by fructose followed by the aqueous extract from Korhogo (Figures 1; 2 and 3). The effects of *Blighia sapida* extracts were contrasted from one region to another due to differences in soils, vegetation and thus climate.

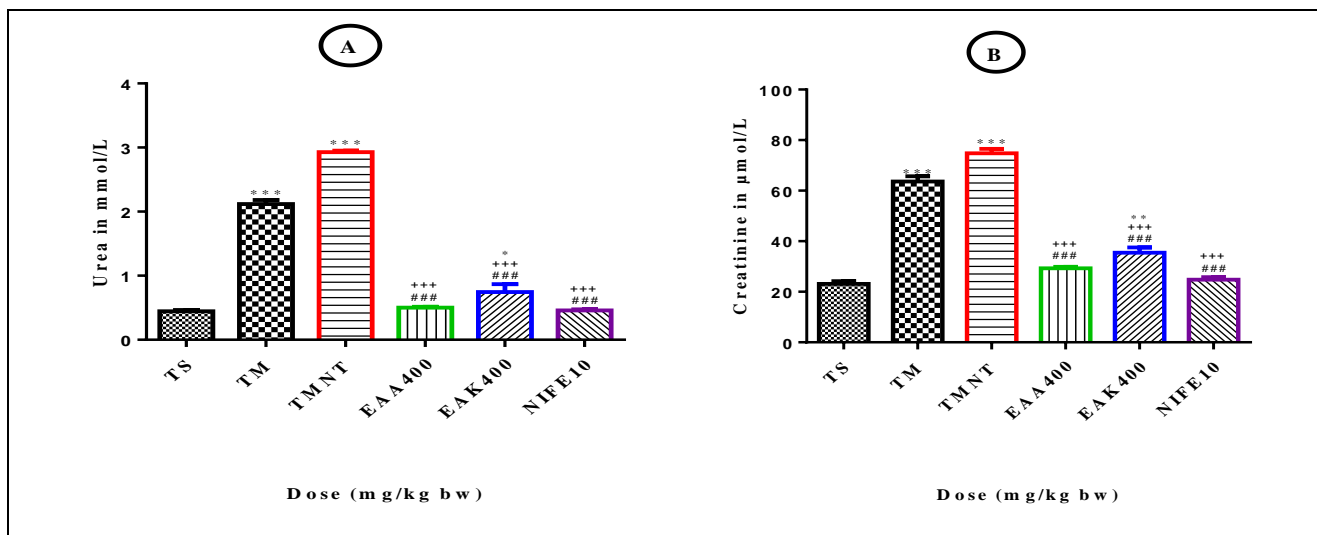


Figure 1: Comparison of the pharmacological effects of EAA and EAK extracts on serum urea (A) and creatinine (B) values in hypertensive rats.

Each bar represents the average \pm SEM. ***, significant difference from the normotensive control lot at $p < 0.001$; * significant difference from the control lot at $p < 0.05$; ###, a significant difference from the hypertensive lot (TM) at $p < 0.001$; +++, a significant difference from the untreated diseased lot (TMNT) at $p < 0.001$; --- significant difference from the Adzopé Aqueous Extract (EAA) lot

TS: normotensive control lot; TM: hypertensive lot; TMNT: untreated diseased lot; EAA 400: lot treated with Adzopé Aqueous Extract at 400 mg/kg bw; EAK 400: lot treated with Korhogo Aqueous Extract at 400 mg/kg bw.

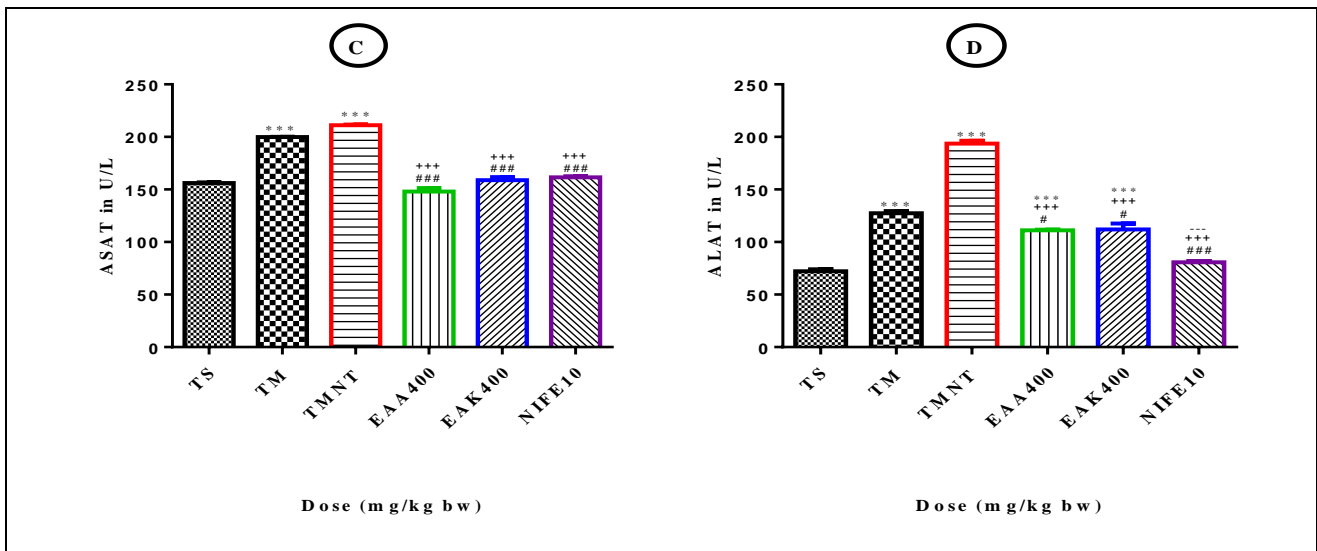


Figure 2: Comparison of the pharmacological effects of EAA and EAK extracts on serum transaminase values: AST (C) and ALT (D) in hypertensive rats.

Each bar represents the average \pm SEM. ***, significant difference from the normotensive control lot at $p < 0.001$; * significant difference from the control lot at $p < 0.05$; ###, a significant difference from the hypertensive lot (TM) at $p < 0.001$; +++, a significant difference from the untreated diseased lot (TMNT) at $p < 0.001$; --- significant difference from the Adzopé Aqueous Extract (EAA) lot

TS: normotensive control lot; TM: hypertensive lot; TMNT: untreated diseased lot; EAA 400: lot treated with Adzopé Aqueous Extract at 400 mg/kg bw; EAK 400: lot treated with Korhogo Aqueous Extract at 400 mg/kg bw.

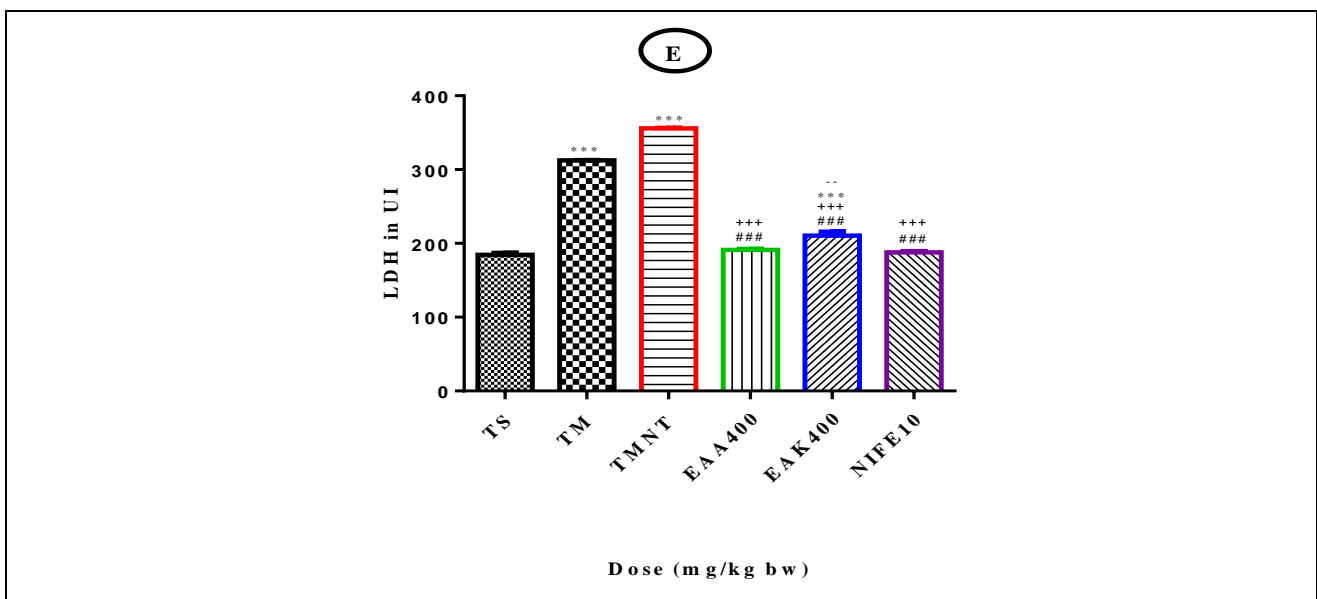


Figure 3: Comparison of pharmacological effects of EAA and EAK extracts on serum lactate dehydrogenase (LDH) values (E)

Each bar represents the average \pm SEM. ***, significant difference from the normotensive control lot at $p < 0.001$; * significant difference from the control lot at $p < 0.05$; ###, a significant difference from the hypertensive lot (TM) at $p < 0.001$; +++, a significant difference from the untreated diseased lot (TMNT) at $p < 0.001$; --- significant difference from the Adzopé Aqueous Extract (EAA) lot

TS: normotensive control lot; TM: hypertensive lot; TMNT: untreated diseased lot; EAA 400: lot treated with Adzopé Aqueous Extract at 400 mg/kg bw; EAK 400: lot treated with Korhogo Aqueous Extract at 400 mg/kg bw.

3.4. Effect of aqueous extracts of *Blighia sapida* on the lipid profile of hypertensive rats

This study showed that consumption of fructose resulted in a significant increase in serum triglyceride (TG), low-density lipoprotein (LDL), total cholesterol (CHOL), and a significant decrease in high-density lipoprotein (HDL) compared to healthy and untreated diseased controls. Figures 4 and 5 show that the administration of aqueous extracts of *Blighia sapida* resulted in a very significant reduction in serum concentrations of TG

(F), CHOL (G), LDL (I) and a significant increase in serum HDL (H) compared to untreated hypertensive rats. These findings were best seen at 400 mg/kg bw with EAA followed by EAK, which had the least effect on lipid disturbances induced by the fructose diet. The effect of aqueous extracts on the lipid profile of hypertensive rats was thus different from the Adzopé region to the Korhogo region and thus from the sub-equatorial (southern) to the Sudanian (northern) climate.

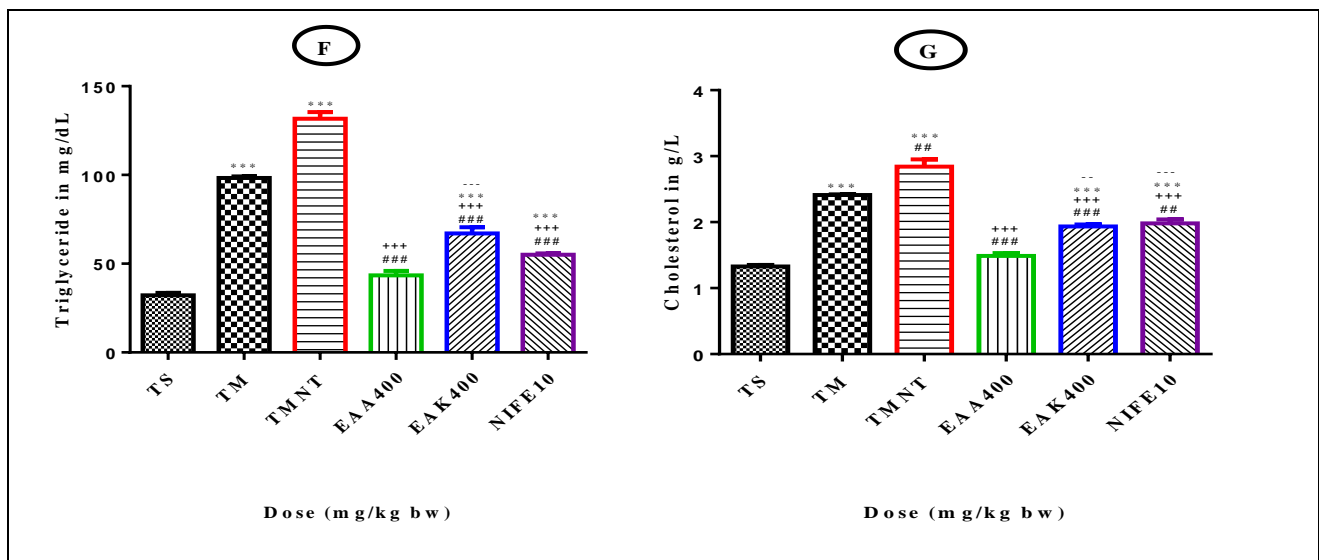


Figure 4: Comparison of the pharmacological effects of EAA and EAK extracts on serum triglyceride (F) and cholesterol (G) values in hypertensive rats.

Each bar represents the average ± SEM. ***, significant difference from the normotensive control lot at p < 0.001; * significant difference from the control lot at p < 0.05; ###, a significant difference from the hypertensive lot (TM) at p < 0.001; +++, a significant difference from the untreated diseased lot (TMNT) at p < 0.001; --- significant difference from the Adzopé Aqueous Extract (EAA) lot

TS: normotensive control lot; TM: hypertensive lot; TMNT: untreated diseased lot; EAA 400: lot treated with Adzopé Aqueous Extract at 400 mg/kg bw; EAK 400: lot treated with Korhogo Aqueous Extract at 400 mg/kg bw.

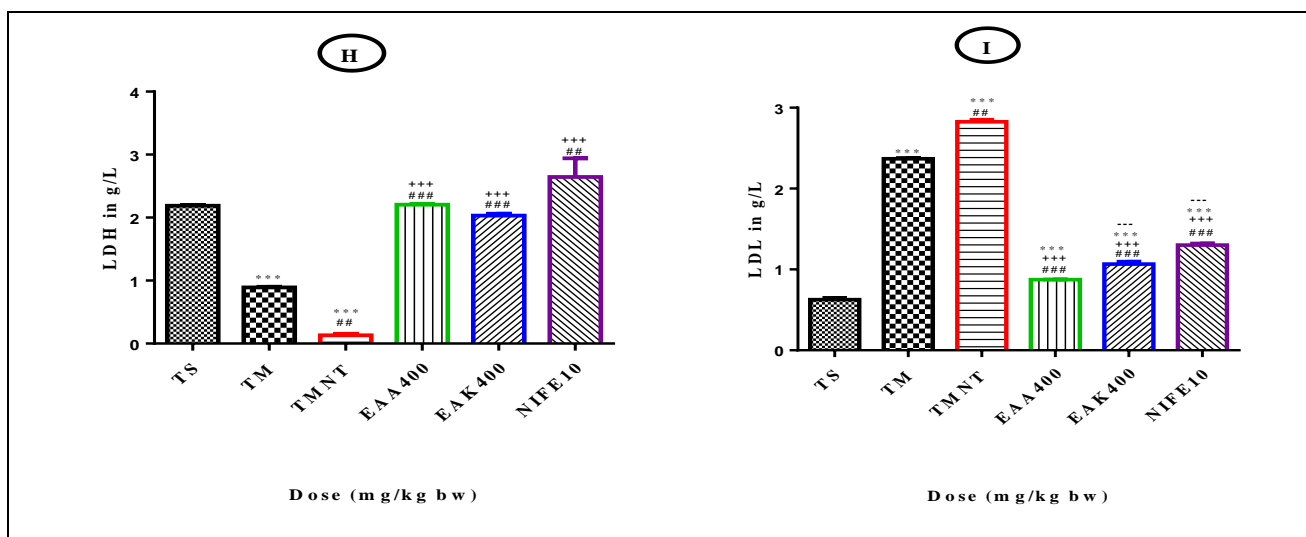


Figure 5: Comparison of Pharmacological Effects of EAA and EAK Extracts on Serum Lipoprotein Values: LDH (H) and LDL (I) in Hypertensive Rats.

Each bar represents the average \pm SEM. ***, significant difference from the normotensive control lot at $p < 0.001$; * significant difference from the control lot at $p < 0.05$; ###, a significant difference from the hypertensive lot (TM) at $p < 0.001$; +++, a significant difference from the untreated diseased lot (TMNT) at $p < 0.001$; --- significant difference from the Adzopé Aqueous Extract (EAA) lot

TS: normotensive control lot; TM: hypertensive lot; TMNT: untreated diseased lot; EAA 400: lot treated with Adzopé Aqueous Extract at 400 mg/kg bw; EAK 400: lot treated with Korhogo Aqueous Extract at 400 mg/kg bw.

4. Discussion

The results of this study showed that hypertension causes a significant and permanent increase in urea, creatinine, AST, ALT, and LDH levels in diseased rats compared to healthy control rats. This increase in serum concentrations of urea and creatinine defines impairment of renal function (Bidié et al., 2016) as well as LDH, which is a marker of cardiac function, indicates myocardial injury (Coulibaly et al., 2010). Also, ALT is a hepatocyte cytosolic enzyme released into the blood in case of necrosis (Nahdi et al., 2018). Their increase in blood indicates hepatotoxicity. Thus, the high values of these enzymes observed in hypertensive rats show that hypertension affects the liver (El Koumbaoui et al., 2017). Aqueous extracts of *Blighia sapida* at a dose of 400 mg/kg bw which normalizes the levels of urea, creatinine, AST, ALT, and LDH in hypertensive rats, would have a hepatic and cardio-protective effect by improving the deterioration of serum markers of the heart and liver. This action of *Blighia sapida* extracts can be attributed to the

antioxidant compounds of the plant such as total polyphenols, flavonoids, or alkaloids. These phytochemicals can stop the increase in transaminases and serum LDH caused by the fructose diet and then reduce their concentration in the blood (Ajiboye et al., 2017). EAA extract at 400 mg/kg bw better normalizes the increased biochemical parameters in hypertensive rats than EAK extract. This effect would be due to its high content of total polyphenols and total flavonoids. These results are in agreement with those of Nahdi et al. (2018) who reported in their study that the extract with the highest total polyphenol content has the highest therapeutic activity.

Dyslipidemia is a potential risk factor for cardiovascular disease. Our study also showed that rats had reduced HDL and significantly increased levels of total cholesterol, triglycerides, and LDL following the work of Geleta et al. (2016). According to these authors, dyslipidemia and hypercholesterolemia are associated with the pathogenesis of high blood pressure induced by the

permanent ingestion of fructose-based diets in rats (Geleta et al., 2016). Treatment of hypertensive rats with 400 mg/kg bw of aqueous extracts of *Blighia sapida* significantly ($P < 0.05$) increased HDL levels and significantly reduced concentrations of total cholesterol, triglycerides, and LDL. These results show the beneficial action of aqueous extracts of *Blighia sapida* on lipid disorders caused by hypertension. Also, the results of Ikumawoyi et al. (2016) explained that extracts from fresh leaves and trunk bark of *Z. zanthoxyloides* significantly reduce serum concentrations of total cholesterol and LDL in rats. This antihyperlipidemic effect of *Blighia sapida* in hypertensive rats could be attributed to flavonoids, tannins, or saponosides. These results corroborate with those of Minato et al. (2003) and Kolawole et al. (2007) who showed that flavonoids reduce LDL and increase HDL in hypercholesterolemic animals and the work of Bilanda et al. (2018) who showed that lipid reduction is one of how medicinal plants exert their hypotensive effect. In addition, tannins are known for their potential to reduce high levels of total cholesterol, triglycerides, and LDL and to restore serum HDL (Ravichandiran et al., 2012; Amani et al., 2016). The effect of *Blighia sapida* aqueous extracts on lipid disturbances caused by the fructose diet was best perceived with Adzopé aqueous extract (EAA) followed by Korhogo aqueous extract (EAK). This could be explained by the very high concentration of total flavonoids and total polyphenols in EAA extract compared to EAK extract. This difference in polyphenol and flavonoid concentrations between extracts would be due to the different climatic conditions in these areas. Indeed, the meteorological and climatic conditions such as the soil, temperature, rainfall, vegetation, and geographical area of the plant influence the synthesis and accumulation of its phytoconstituents (El Hazart et al., 2015; Allam and Ayad, 2015; Evenamede et al., 2017).

Conclusion

The present study shows that fructose consumption induces hypertension followed by an increase in serum markers of the kidney, heart, and liver and then lipid profile in rats. The treatment of animals with aqueous extracts of *Blighia sapida* from the South of Ivory Coast normalizes these parameters as well as possible as the aqueous extract of the same plant harvested in the North of Ivory Coast. This difference in the efficiency of the plant would be due to the different climatic conditions of the two harvesting areas of the plant. This explains the use of the plant in traditional medicine in some areas of the Ivory Coast compared to other areas where its therapeutic effects are unknown.

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