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Evaluation of the Safety and Efficacy of Extracts from the Leaves of Five Plants Used for the Treatment of Arterial Hypertension in Benin

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Abstract

Cardiovascular diseases (CVD) are nowadays real health problems in the world. High blood pressure is one of the most important risk factors for CVD and is affecting more and more people in Benin. The objective of our work is to evaluate the safety and the efficacy of the leaves of five plants used for the treatment of hypertension in Benin. Acute toxicity was evaluated on wistar rats which orally administered a single dose of 2000 mg/kg of body weight of hydro-ethanolic extract of the leaves of *Phyllanthus amarus* Schumach. & Thonn., *Persea americana* MILL., *Ipomoaea fistulosa* Mart. ex Choisy., *Heliotropium indicum* L., *Schrankia leptocarpa* DC., and were monitored over a period of 14 days. Subacute toxicity was evaluated on rats which received a daily dose of 200 mg/kg of body weight of the plant leaf extract over a period of 28 days. Plant efficacy was assessed by measuring potassium in plant leaves. Administration of the single dose of the extract did not cause any deaths in rats; the weight of the rats varied depending on the extracts administered. Concerning the subacute treatment, the levels of aspartate amino transaminases (AST) and Alanine amino transaminases (AST) did not vary significantly after the 28 days of treatment with the different extracts. On the other hand, a significant increase in serum creatinine was observed in rats treated with extracts of *Phyllanthus amarus*. The leaves of *Heliotropium indicum*, *Ipomoaea fistulosa* and *Phyllanthus amarus* contain the highest levels of potassium. Among the five plants studied, only the leaves of *Phyllanthus amarus* seem to induce renal toxicity. Extracts from the leaves of *Heliotropium indicum*, *Ipomoaea fistulosa* might be the most effective in inducing hypotensive activity and do not show toxicity.

Keywords

Hypertension, Plant Extracts, Toxicity, Efficacy, Potassium

1. Introduction

Arterial hypertension is defined by the WHO as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg. The number of adults aged 30 to 79 with hypertension has increased from 650 million to 1.28 billion over the past thirty years (1990 to 2019). In Africa its prevalence is estimated at 46% and in Benin, the prevalence of hypertension is estimated at 27.5% [1] [2] [3]. One of the complications of hypertension is atherosclerosis which is a multifactorial chronic disease initiated by the retention of oxidized lipoproteins in the arterial wall and accompanied by an inflammatory state, oxidative stress and dysfunction of the vascular endothelium [4]. Herbal medicines have basically been used for health care all over the world since the earliest days of the human species. They are still widely used and have considerable importance in international trade. Recognition of their clinical, pharmaceutical and economic value continues to grow, although this varies greatly by country [5]. In Benin, where 80% of the population has recourse to traditional medicine, this constitutes an alternative to the needs of the populations in terms of health care in the face of an underdeveloped health system. Traditional medicine is very old. It is the sum of all the knowledge, skills and practices based on the theories, beliefs and experiences specific to different cultures, whether explicable or not, and which are used in the preservation of health, as well as in the prevention, diagnosis, amelioration or treatment of physical or mental illness [6]. For many millions of people, herbal medicines, traditional treatments and traditional practitioners are the main, if not the only, source of health care. This care is close to the population, easy to access and financially affordable. They are culturally acceptable and a large number of people trust their virtue. The affordability of most traditional medicines makes them all the more attractive at a time when healthcare costs are blowing up and austerity is nearly universal. Traditional medicine also appears as a response to the inexorable rise of chronic non-communicable diseases [7]. Accessibility, whether geographical, cultural or financial, is positioned as one of the major obstacles to the use of modern healthcare [4]. The taking of medicinal plants can be done in different types. These can be capsules, tablets, infusions, tinctures, extracts, raw herbs or in different forms including enemas or poultice applications. Many researchers are turning to the ethno pharmacological approach to find new active natural derivatives in order to develop a scientific basis for the preventive and therapeutic use of plants. Research on traditional medicines is needed to monitor their quality, safety and efficacy. The use of products whose quality, safety and efficacy have not been verified represents a real danger because of the side ef-

fects and consequences that these drugs could have on certain organs such as the liver and the kidney, inducing hepatotoxicity or renal toxicity [8] [9] [10]. Plant toxicity studies highlight the toxic effects of plant extracts with established biological properties [11]. Thus, before examining the therapeutic activity of a drug or its constituents, it is necessary to know its harmlessness through the study of toxicity.

In this work, we evaluated the toxicity and the efficacy of the extracts of the leaves of five plants selected at the end of an ethno pharmacological study carried out on the plants which treat the arterial hypertension in Benin.

2. Materials and Methods

2.1. Vegetal Material

It consists of the leaves of five medicinal plants (*Phyllanthus amarus*, *Persea americana*, *Ipomoaea fistulosa*, *Heliotropium indicum and Schrankia leptocarpa*) selected for their use in the treatment of hypertension in some communes of Benin. The plants were selected after an ethnopharmacological study considering their frequency of use by traditional healers and the existence of studies already carried out on the plants [12]. They have been identified and authorized in the national herbarium of the University of Abomey Calavi. After harvesting, the samples were dried at laboratory temperature until their plant mass stabilized and then reduced to powder.

2.2. Animal Material

The experimental animals are male and female Wistar rats weighing between 150 and 250 g. All animals have health status of SPF (specific pathogen Exempt). Work on wistar rats were authorized by the national committee of ethics of Benin science academy. Upon receipt, the rats were randomly placed in groups of five (5) in standard cages for a period of acclimatization (2 weeks) before being used in various experiments. During this period the animals had free access to food and water and remained kept at constant temperature (22 ± 2)˚C. They were subjected to a light/dark cycle 12 h/12 h. The dark phase of the cycle begins at 12 h and different experiences have always been held from 11AM to 6PM due to the nocturnal activity (active phase) of rats.

2.3. Preparation of Hydroethanolic Extracts

Ethanol and water were used as extraction solvents. For the hydro-ethanolic extraction, a quantity of 50 g of powder was macerated in 500 mL of the two solvents, *i.e.* 250 mL of distilled water and 250 mL of ethanol. The mixture was subjected to light mechanical stirring for 24 hours at room temperature. After filtration, the extracts were lyophilized to obtain the dry crude extracts.

The yield was calculated using the following formula:

$$\text{Yield } (\%) = m/M \times 100$$

m = mass of the prepared extract; M = mass of the powder used.

2.4. Assessment of Acute Toxicity

The acute oral toxicity test was performed according to the guidelines of the Organization for Economic Co-operation and Development (OECD) [13]. The rats were fasted for 4 hours with free access to water. They were randomly divided into 6 groups of three rats and treated orally with a single dose of hydroethanolic extract of the leaves of the five plants (2000 mg/Kg) respectively or distilled water (20 ml/Kg) used as control. After the treatment, the rats were monitored and observed individually every hour for 4 hours and then every day for 14 days. An information sheet was produced for each group of rats in order to collect possible signs of toxicity (mortality, weight, changes in the skin, hair, eyes, somatomotor activity and behavior).

2.5. Assessment of Subacute Toxicity

The subacute oral toxicity test was performed according to the 407 guidelines of the Organization for Economic Co-operation and Development (OECD). It was carried out on 18 albino Wistar rats divided into six equal groups of 3 as follows: group 1, receiving daily distilled water at a rate of 1 ml/100 mg of body weight (control group); batches 2, 3, 4, 5 and 6 receiving daily a solution of the hydro-ethanolic extract of the leaves of the five plants at a rate of 200 mg/kg of body weight respectively for each sample. The treatment lasted 28 days. Rats were fed and hydrated freely, then weighed every 14 days. A blood sample was taken before and at the end of the treatment. Several biochemical parameters were assayed in particular; creatinine by kinetic method (BIOLABO) kit [14]. Alanine aminotransferase by ALT GTP (IFCC) Single vial (BIOLABO) kit and aspartate aminotransferase by AST GOT (IFCC) Single vial (BIOLABO) kit [15].

2.6. Potassium Assay [16] [17]

- Determination of organic matter and ash

The method adopted is that often used for the determination of organic matter in sediments. It consists of calcining the sample at 450˚C for 3 hours, (without pre-drying at 103˚C ± 2˚C) the observed mass loss is attributed to organic matter and therefore represents the mass percentage of organic matter, then preparation of the sample solutions.

- Atomic absorption spectrophotometer (AAS) assay

Procedure: The wavelengths of the elements to be analyzed are first defined on the device. Then, the different readings of the calibration ranges permit to establish the calibration curve reflecting the absorbance as a function of the concentration. Finally, the solutions containing the ash are presented to the apparatus in order to determine the absorbance.

2.7. Statistical Analyzes

The data (variation in weight, transaminases, creatinine) collected in the rats before and after treatment with the different plant extracts were entered into

Excel software. R software was used for testing. Thus, statistical inference, the normality test (Ryan Joiner test) was used to verify the normality of the data. Levene's test was used to check the homogeneity of the variances with $P < 0.05$.

3. Results

3.1. Yield of Extractions

The yields obtained varied from one plant to another. They are between 12.6 and 18.2%. *Persea americana* and *Ipomoaea fistulosa* had the best yields (Table 1).

3.2. Assessment of Acute Toxicity Change in Weight of Rats during the Acute Toxicity Test

Evolution of the weight of the rats

From the analysis of the results (Figure 1), we notice that there are no significant variations in weight in the rats which received the extracts of Phyllanthus amarus, Persea americana and Schrankia leptocarpa and Ipomoaea fistulosa. But a significant decrease (P value = 0.0315 < 0.05) in rats that received the extract of Heliotropium indicum. A significant increase was observed in control rats (P value = 0.015 < 0.05).

Mortality and other signs of toxicity

No mortality was recorded in the first hours after administration of the single

Table 1. Yield of hydroethanolic extraction of each sample.

Samples	Yields (%)
Phyllanthus amarus	16.2
Persea americana	18
Ipomoaea fistulosa	18.2
Heliotropium indicum	14.4
Schrankia leptocarpa	12.6

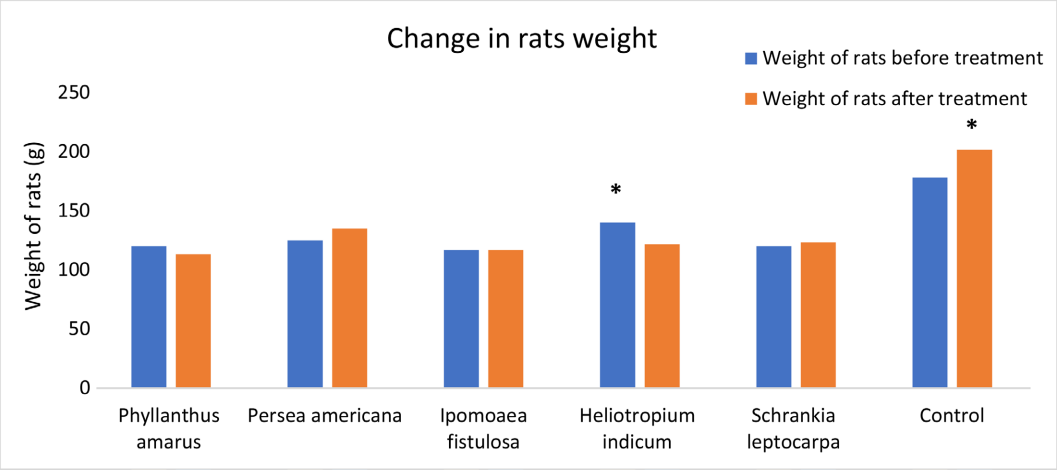

Figure 1. Weight change of rats during acute toxicity test (*P < 0.05).

dose of 2000 mg of extracts of the leaves of the five plants to rats. On the other hand, the treated rats showed a general weakness compared to the control rats. After 14 days of observation, no death was observed in the treated rats. In addition, no other signs of toxicity such as tremor, reaction to noise, change in coat were observed (Table 2).

3.3. Assessment of Subacute Toxicity

Evolution of the weight of the rats during the treatment

The rats which received the extracts of *Phyllanthus amarus*, *Persea americana* and *Schrankia leptocarpa* did not experience significant variations in their weights 14 days and 28 days after the treatment. A significant increase was observed in rats that received *Ipomoaea fistulosa* 28 days after treatment with P value = 0.005 < 0.05. A significant increase was observed 14 days after treatment with rats treated with *Heliotropium indicum* leaf extracts, with a P value = 0.04 < 0.05 (Figure 2).

Variation in Alanine Transaminase (ALT) level in treated rats

Before the treatment (D = 0), the levels of Alanine transaminases (ALT) are abnormally high in all the rats, including the controls. After treatment, a decrease

Table 2. Effect of single dose of extracts on mortality and other physiological parameters in rats.

group	Hydroethanolic extract	Number of deaths	Tremor	Abnormal motility	Reaction to noise	Coat change
1	*Phyllanthus amarus*	0	No	No	No	No
2	*Persea americana*	0	No	No	No	No
3	*Ipomoaea fistulosa*	0	No	No	No	No
4	*Heliotropium indicum*	0	No	No	No	No
5	*Schrankia leptocarpa*	0	No	No	No	No

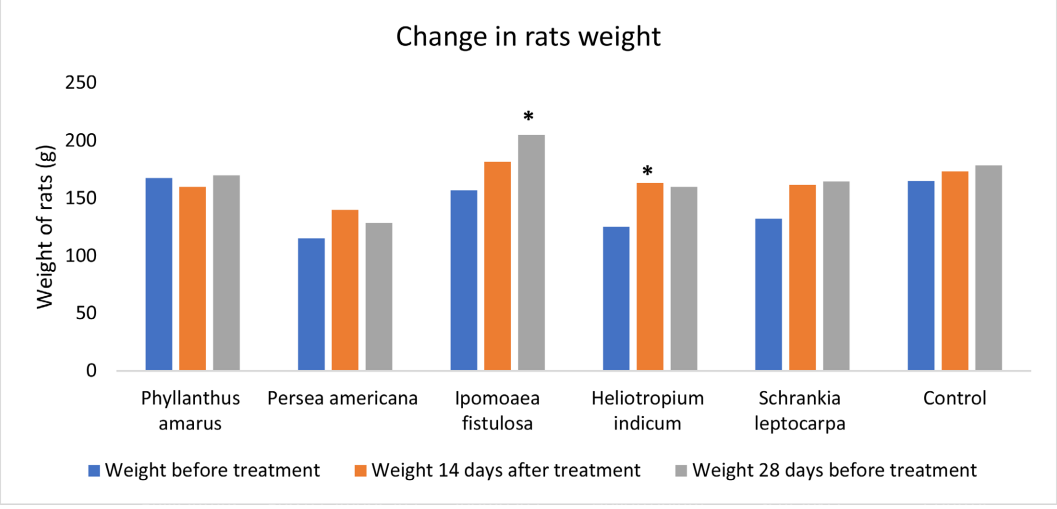

Figure 2. Variation of rat weights 14 and 28 days after treatment with extracts (*P < 0.05).

in levels was observed in all rats with a significant decrease in control rats. No plant extract induced an increase in ALT transaminase levels in treated rats (Figure 3).

Variation in Aspartate transaminase (AST) level in treated rats

A non-significant decrease in the Aspartate transaminase level was observed in the rats treated with the hydroethanolic extracts of the leaves of the five plants after 28 days of treatment. The AST level remained nearly constant in control rats that received distilled water (Figure 4).

Variation in Creatinine level in treated rats

We did not note any significant variation in the level of creatinine in rats treated with the hydroethanolic extract of *Persea americana*, *Ipomoaea fistulosa*, *Schrankia leptocarpa*, *Heliotropium indicum* as well as in control rats. However, a significant increase was observed in rats treated with Phyllanthus amarus extract (P-value = 0.028 < 0.05) (Figure 5).

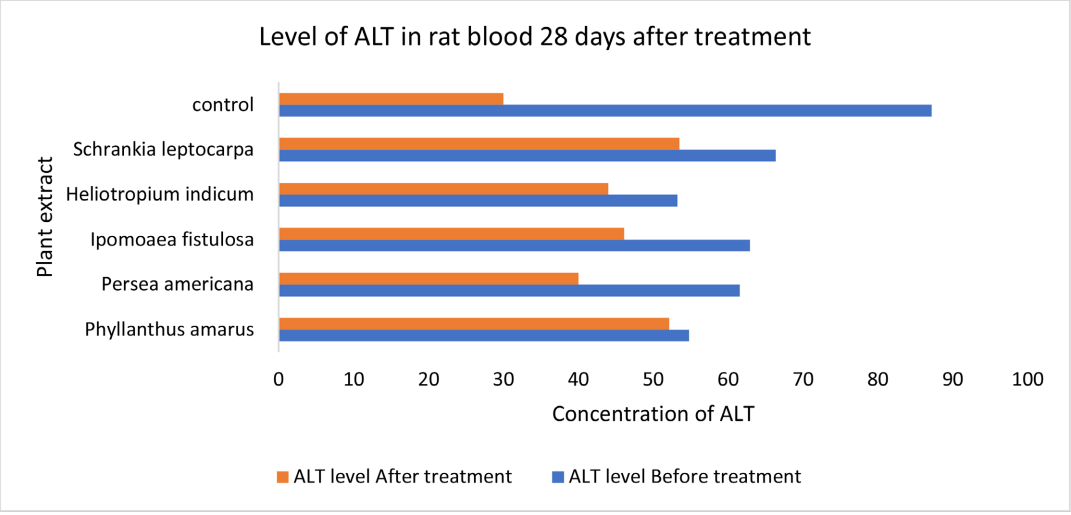

Figure 3. Variation of Alanine transaminase (ALT) in treated rats blood.

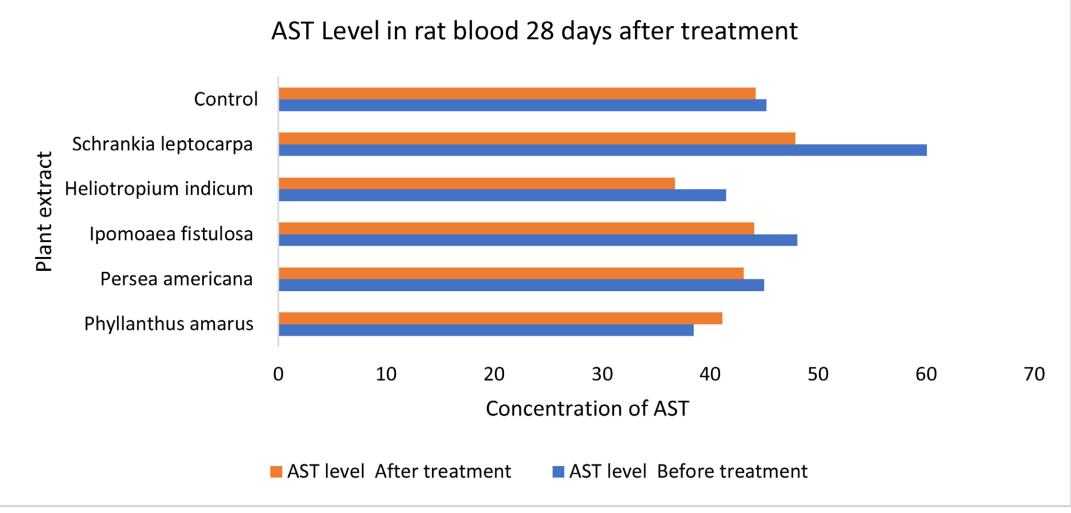

Figure 4. Variation of Aspartate transaminase (AST) in treated rats blood.

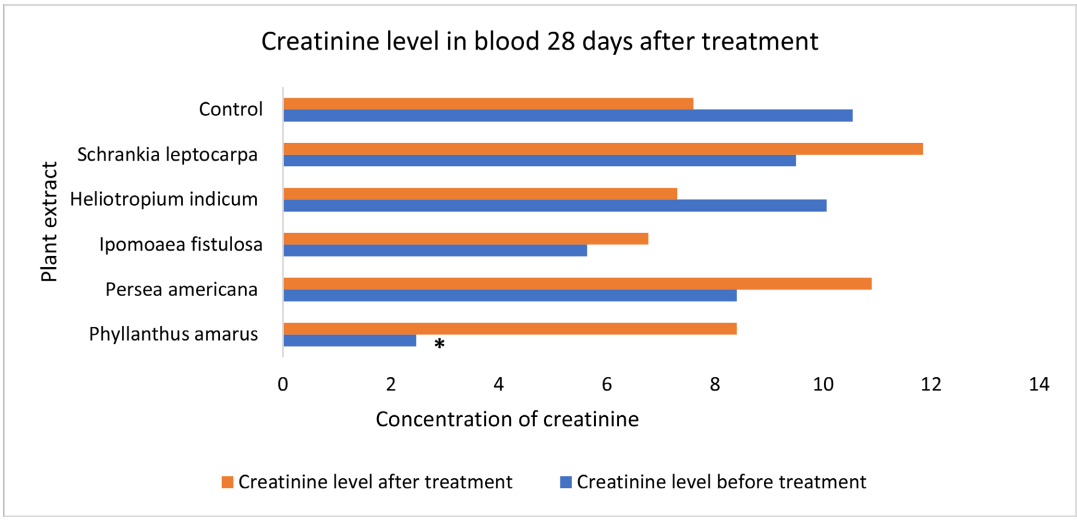

Figure 5. Variation of creatinine level in treated rat blood (*P < 0.05).

Table 3. Result of the potassium content of each plant leaves.

Plants	Potassium content mg/100 g
Phyllanthus amarus	1543.75
Persea americana	784.94
Ipomoaea fistulosa	2411.33
Heliotropium indicum	3462.84
Schrankia leptocarpa	917.78

3.4. Evaluation of Plant Efficacy by Potassium Assay

Potassium plays an important role in regulating blood pressure. So to check the efficacy of these plants used by traditional healers for the treatment of hypertension, we measured the potassium content of our different plants. Analysis of the assay results shows that the leaves of *Heliotropium indicum* are richer in potassium, followed by the leaves of *Ipomoaea fistulosa* and then the leaves of *Phyllanthus amarus*. The leaves of *Schrankia leptocarpa* and *Persea americana* are the least rich in potassium (Table 3).

4. Discussions

Herbal Safety: Acute Toxicity

Changes in body weight, food intake and changes in general behavior are important because they are the first signs of toxicity [18] [19]. The body weight of the rats treated with 2000 mg/kg of leaf extracts of *Persea americana*, *Schrankia leptocarpa*, *Phyllanthus amarus and Ipomoaea fistulosa* did not vary contrary to the rats controls which were not treated with the extracts. In addition, a significant decrease was observed in rats that received the *Heliotropium indicum* extract. The decrease and constant weight observed in the treated rats could be linked to the loss of appetite induced by the treatments [20]. The toxicity of a

substance can be defined as its ability to produce harmful effects in a living organism. It varies according to the dose, the frequency, the duration of exposure, and the time of appearance of clinical signs. Any substance intended to be placed on the market, whether it is a drug or a chemical, must undergo three types of toxicity tests to assess its safety [21]. The signs of toxicity have not been observed and the unrecorded death of the rats during the fourteen (14) days of observation show that the hydro ethanolic extracts of our various plants administered orally up to a maximum dose of 2000 mg/kg are devoid of acute toxicity in wistar rats under the conditions of our study. The chemical components of our samples therefore appear to be non-toxic.

Subacute toxicity

The weight of rats treated with extracts from the leaves of *Persea americana*, *Schrankia leptocarpa* and *Phyllanthus amarus* did not vary significantly during the 28 days of treatment. However, the weight of the rats treated with the extracts of the leaves *of Ipomoaea fistulosa* and *Heliotropium indicum* increased significantly either on the 14th day or on the 28th day. These results show that rats react differently to different treatments. They tolerated the extracts of the last two plants faster than the others which did not seem to induce a reduction in their weight. The assays carried out showed no significant increase in AST and ALT transaminases in the rats treated for 28 days with the dose of 200 mg/kg of body weight. ALTs are more specific for liver damage, while ASTs increase with haemolysis [22]. The liver is a target organ of xenobiotics and plays a role in the detoxification process. An increase in the concentration of the enzyme AST and ALT indicates poor functioning of the liver due to intoxication. The modification of these biochemical parameters due to toxic substances represents an important index and can constitute a diagnostic tool in toxicological studies [23] [24]. The absence of significant variation of these enzymes in the treated rats suggests that the hydroethanolic extracts of our plants have no hepatotoxic effect. In addition to the liver, the kidney plays an important role in the body's homeostasis, ensuring the filtration of toxic waste from the bloodstream and its excretion in the urine [25]. Our results showed a non-significant variation of creatinine level in the treated rat blood except the rats which received the hydro-ethanolic extract of the leaves of *Phyllanthus amarus* in whom we noted a significant increase in the level of creatinine. Creatinine measures kidney filtration. Usually it is filtered by the kidneys with little or no tubular reabsorption [26]. Its high level in the blood reflects a decrease in glomerular filtration and therefore renal failure. The extracts of *Persea americana*, *Schrankia leptocarpa*, *Ipomoaea fistulosa* and *Heliotropium indicum* which did not induce a significant variation in the creatinine level can be considered as non-toxic for the kidneys, whereas the extracts of *Phyllanthus amarus* could induce renal toxicity.

Efficacy of plants: Dosage of potassium, an important mineral in the regulation of blood pressure

The analysis of our results shows that the leaves of *Heliotropium indicum*, *Ipomoaea fistulosa and Phyllanthus amarus* are the richest in potassium. Potas-

sium plays several roles in the body including the regulation of blood pressure. Potassium is the main intracellular cation with an intracellular concentration of 130 to 150 mmol/l and an extracellular concentration of 3 to 5 mmol/l in adult humans. This high cell concentration is maintained by the Na-K-ATPase pump. In the case of essential hypertension, due to the increase in the activity of the sodium pump, a diet rich in sodium increases kaliuresis (by exchange of sodium and potassium cations) at the level of the renal collecting duct. Potassium depletion in turn increases sodium retention by stimulation of the Na^+/H^+ exchanger (via intracellular acidosis and stimulation of the sympathetic nervous system and the renin-angiotensin system) which builds a vicious circle [27]. Conversely, a potassium-rich diet in hypertensive patients will induce natriuresis, with consequent lowering of arterial pressure, which is most likely explained by deactivation of the distal tubule Na^+/Cl^- cotransporter (aldosterone-independent), a decrease in intracellular calcium and vasodilation (mechanisms which would explain the antihypertensive action of thiazide diuretics). Potassium also exerts a direct effect on the arterial wall which could intervene in the regulation of blood pressure, in particular through the vasodilation induced by the hyperpolarization of smooth muscle cells. This hyperpolarization will take place by the release of potassium through the opening of potassium channels dependent on the intracellular calcium concentration. Potassium would also have the ability to scavenge norepinephrine at the nerve endings and therefore reduce its effectiveness as a vasoconstrictor and therefore the reduction of blood pressure [28] [29] [30]. Thus a diet rich in potassium lowers blood pressure and reduces the risk of stroke and heart disease [31] [32].

5. Conclusion

The toxicological study of the five plants carried out by the evaluation of the acute and subacute toxicity showed an absence of acute toxicity of the five plants. However, the subacute toxicity study showed that among the five plants *Phyllanthus amarus* could induce renal toxicity. The evaluation of the efficacy through the dosage of potassium in the plants revealed that the leaves of *Heliotropium indicum*, *Ipomoaea fistulosa* and *Phyllanthus amarus* are the richest in potassium. Considering our work we can deduce that *Heliotropium indicum* and *Ipomoaea fistulosa* are plants that could treat high blood pressure without risk of toxicity.

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Source

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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