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Research Article

# Evaluation of organic acids, cytotoxicity, oral acute toxicity and diuretic effect of Phaseolus vulgaris crops ethyl acetate extract for cardiovascular health improvement

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## Abstract

Diuretics play an important role in managing high blood pressure. The aim of this study was to determine the organic acids, toxicity, and diuretic properties of the ethyl acetate fraction of *Phaseolus vulgaris* (EPV) in Wistar rats. High Performance Liquid Chromatography (HPLC) analysis has been used to detect and quantify the organic acids in the EPV fraction. Cytotoxicity tests of *Artemia salina* larvae and acute oral toxicity tests are carried out to determine the lethal concentrations and doses of EPV. The diuretic properties of EPV were evaluated in Wistar rats for 24 hours by measuring diuresis parameters and urinary electrolyte concentrations.

**Results**: The HPLC analysis report showed the presence of various organic acids in EPV, including malic acid (117.79±0.01 mg/g), oxalic acid (52.47±0.00 mg/g), and citric acid (42.85±0.00 mg/g). EPV seems almost non-toxic, as demonstrated by the larvicidal death lethal concentration ($LC_{50}$) and the estimated 50% lethal dose of 5000 mg/kg body weight. Measuring diuresis parameters reports that EPV may increase urine volume at the test doses. In addition, the report states that EPV affects electrolyte balance by increasing the excretion of $Na^+$, $K^+$, $Cl^+$, and pH. The intensity of the diuretic effect was significantly higher than that of the negative control. These effects are less pronounced than those caused by pharmacological references but are largely similar to those caused by furosemide. A high concentration of oxalic acids, citric acids and ascorbic acids, combined with the proven urine effects of EPV, strengthens the antihypertension, antioxidant and vasorelaxant properties previously reported. These drug properties demonstrate not only the traditional use of EPV but also its use as a database for the development of innovative therapeutic tools to reduce the risk of cardiovascular diseases.

**Keywords**: Diuretic effect; Organic acids; *Phaseolus vulgaris* ethyl acetate fraction

## INTRODUCTION

Over the years, diuretics have been widely used as the first line of treatment for high blood pressure combined with other drugs. Diuretic drugs are a heterogeneous family of drugs whose main effect is to increase the blood flow regulating by modulating the ion balance of the electrolytes (sodium, potassium, and chlorides) in the kidney (1). Based on their objectives and mode of action, the drug family can be classified into urine for carbon dioxide inhibitors, urine for osmotics, urine for loops, and urine for potassium conservation (2). Among these categories of diuretics, thiazides are widely used alone or in combination with potassium-sparing diuretics, depending on the patient's clinical condition. The effectiveness of these diuretics in reducing blood

pressure and reducing the risk of cardiovascular disease has been widely documented (3,4). Furthermore, these diuretics have an antihypertensive efficacy comparable to an enzyme inhibitor that converts angiotensin (4). In addition to its use in cardiology, epidemiological data show that diuretics are highly used in hospital and outpatient care to treat hypertension and edema. Given the many side effects associated with the long-term use of thiazide diuretics, new alternative and complementary strategies would be an important asset for the development of diuretic resistance (5, 6). In fact, high levels of thiazides induce electrolytes imbalances and lead to hypokalemia and hypotension, which in most cases leads to arrhythmia, reduced glucose tolerance, possible reduction in insulin secretion, and erectile dysfunction (7–9). Consequently, the discovery and development of natural diuretics are a promising option. Medicinal plants contain various bioactive molecules, and their synergistic effects effectively contribute to the management of several symptoms (10). Our previous work on *Phaseolus vulgaris* established an antioxidant, vasorelaxant effect on the thoracic aorta and an antihypertensive effect on hypertensive mice following oral administration of LNAME (11, 12). This article aims to evaluate the organic acid profile, the diuretic effect, larval cytotoxicity, and acute oral toxicity of the ethyl acetate fraction (EPV).

## 1. MATERIAL AND METHODS

### 1.1 Material

#### 1.1.1 Vegetal Material

*Phaseolus vulgaris* crops were harvested in the village of Loumbila, about 18 km from Ouagadougou, Burkina Faso. The raw materials collected were dried at ambient temperature, ventilated, covered with sunlight, and finely ground using a grinder. The powder obtained was kept at room temperature until extraction (12).

#### 1.1.2 Animal material

Male Wistar rats weighing 136±6 g, supplied by the Institute for Health Sciences Research (IRSS), were used in the study. These animals were housed in accordance with acceptable animal husbandry practices, with free access to food (29% protein) and tap water. The experiment was conducted in strict compliance with the standard operating procedures defined by the Declaration of Helsinki on best practice in animal experimentation (13).

#### 1.1.3 Solvent and reagent

Furosemide, amiloride, and hydrochlorothiazide were used as pharmacological reference substances. $NaH_2PO_4$ was provided by Merck (Germany), and water was purified with a Direct-Q UV system by Millipore (USA). The pure standards of oxalic, malic, ascorbic, citric, succinic, and fumaric acids (purity 99% HPLC) were purchased from Merck, Germany.

### 1.2 Methods

#### 1.2.1 Extraction

The dry powder of *P. vulgaris* crops was decocted in a ratio of 01 g to 05 mL of distilled water. The clear decoction obtained was freeze-dried and then fractionated with ethyl acetate. The ethyl acetate fraction was concentrated using a rotavapor and dried in an oven at 40°C. EPV refers to the dry powder derived from *P. vulgaris'* ethyl acetate fraction.

#### 1.2.2 HPLC-VWD analysis for organic acids of EPV

0.1 g of EPV powder was boiled in 01 mL distilled water, then treated with a 30 min ultrasonic at room temperature, then supernatant, containing the extracted organic acids, filtered by a nylon filter (0.45 µm diameter), and 20 µL was injected into the HPLC system. Analysis was carried out using an HP-1200 liquid chromatograph equipped with a quaternary pump, manual injector, and VWD detector (Agilent Technologies, USA). The column was an Acclaim OA (5 µm; 4x150 mm i.d.) from Thermo Fisher Scientific, USA. The mobile phase was $NaH_2PO_4$, 50 mM solution at pH=2.8; elution was done for 10 min at room temperature with a flow rate of 0.5 mL/min. Chromatograms were recorded at wavelength λ=210 nm, and data acquisition was done with the Agilent ChemStation software.

#### 1.2.3 Determination of larval cytotoxicity of the EPV fraction

The experimental method of Vanhaeeke et al. (1981), adapted by Akadiri, was applied (14,15). The EPV fraction's starting solution (50 mg/mL) was generated using seawater. Successive dilutions were carried out to generate a decreasing range of concentrations of 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.12 mg/mL, 1.56 mg/mL, 0.78 mg/mL, 0.39 mg/mL, 0.19 mg/mL, 0.09 mg/mL, and 0.04 mg/mL. Brine shrimp larvae (*Artemia salina* L.) obtained by cultivating eggs in seawater were put in each tube in groups of sixteen (15). The test tubes were expected following a 24-hour incubation period at laboratory temperature. The number of dead larvae was counted. A control tube was prepared with larvae in seawater without extract for comparison. This experiment was performed in triplicate. The lethal concentration ($LC_{50}$) was then determined. EPV's cytotoxicity was assessed using the Mousseux scale (16).

#### 1.2.4 Acute oral toxicity

This trial was conducted in accordance with OECD Guideline 423 (17). Healthy mice of similar weight were fasted and placed into two groups of three mice each (control and EPV). First, the test group received a single oral dose of 2000 mg/kg of EPV, while the control group received 0.9 % NaCl. The mice were then observed ethologically, and clinical signs of toxicity were reported every 30 minutes for two hours. Food and water were then provided *ad libitum* throughout the study (14 days). At the end of the study, the mice were humanely

sacrificed, and the vital organs (liver, spleen, kidneys, lungs, and heart) were removed, observed, and weighed. All steps were repeated under the same conditions.

### 1.2.5 Determination of the diuretic activity of EPV

Diuretic activity was assessed using the approach described before (18, 19). Male rats of similar weight were appropriately acclimatized and fasted for 12 hours before being separated into six groups of five rats each. Group I (control) received physiological saline (0.9% NaCl) at a dose of 25 mL/kg. Groups II, III, and IV, which served as references, received furosemide (15 mg/kg), amiloride (5 mg/kg), and hydrochlorothiazide (25 mg/kg), respectively. Groups V and VI received EPV dosages of 10 and 20 mg/kg, respectively. Following treatment, each rat was placed in a metabolic cage and fasted for 24 hours. Urine was collected in a graduated tube, and the volume was measured after three (03) hours, six (06) hours, and twenty-four (24) hours. Sodium ($Na^+$), potassium ($K^+$), and chloride ($Cl^-$) ions were measured using an electrolyte analyzer. In addition, the pH of each urine sample was monitored at regular intervals. Urine volume or urine output, natriuretic, and salidiuretic indicators were determined.

### 1.2.6 Statistical analysis

All results are expressed as averages with standard deviations and are plotted with GraphPad Prism 8.0 software (GraphPad Software, San Diego, California, USA). The variance analysis was followed by the Bonferroni test, with a p-value under 0.05 considered statistically significant.

### 1.2.7 Ethics considerations

All research and experiments carried out in the environment are carried out in accordance with protocols already validated by the Institut de Recherche en Sciences de la Santé (IRSS), which comply with international standards (European Union Animal Protection Guidelines CCE Council 86/609).

## 2. RESULTS

### 2.1 Determination of organic acid content

The chromatogram below shows the profile of the standard and each peak corresponding to a well-defined organic acid (Figure 1). For example, the peak observed at a retention time of 03 min is oxalic acid, while the peak at 4 min corresponds to succinic acid.

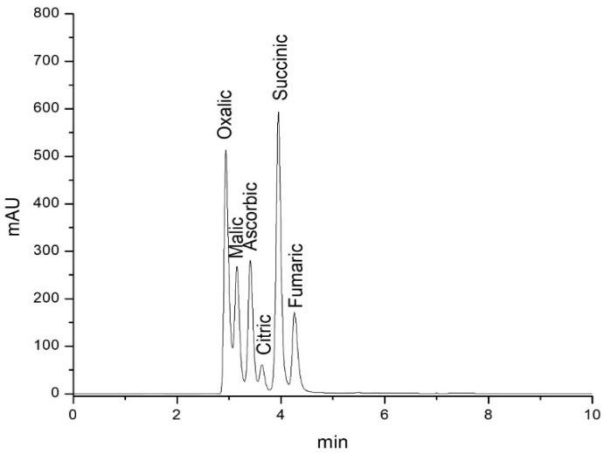



Figure1: Chromatogram of organic acid standard

*Note: 1= oxalic acid (Rt=2.94 min); 2= malic acid (Rt=3.15 min); 3= ascorbic acid (Rt=3.34 min); 4= citric acid (Rt=3.69 min); 5= succinic acid (Rt=3.94 min); 6= fumaric acid (Rt=4.38 min)*

Figure 2 shows the respective chromatograms of the organic acids in the EPV fraction obtained by HPLC. Analysis of the figure reveals the presence of oxalic, malic, ascorbic, citric, succinic, and fumaric acids in peaks 1, 2, 3, 4, 5, and 6, respectively.

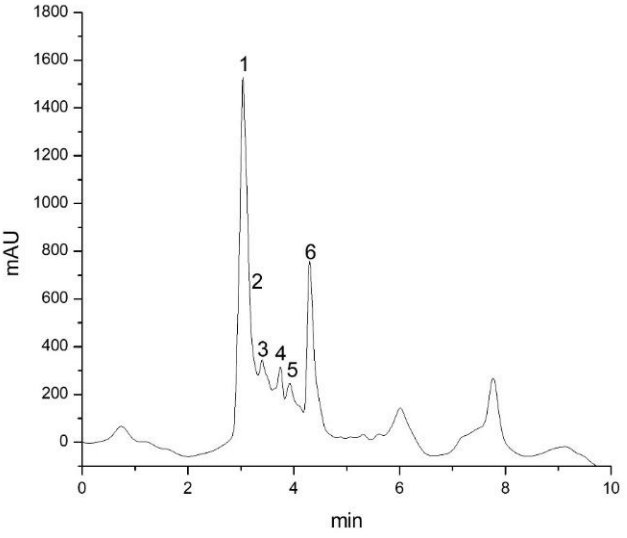



Figure 2: Chromatogram of organic acids in the ethyl acetate fraction of *P. vulgaris*.

*Note: 1= oxalic acid; 2= malic acid; 3= ascorbic acid; 4= citric acid; 5= succinic acid; 6= fumaric acid*

Table I below summarizes the organic acids and their concentration expressed in mg/g of dry extract. Analysis of the table shows that malic and oxalic acids are the most abundant unsaturated fatty acids in EPV. Although citric, ascorbic, and succinic acids are not the most abundant, they still account for a significant proportion. The concentration of fumaric acid is very low in EPV.

Table I: Organic acid content of ethyl acetate fraction of *P. vulgaris* (EPV)

| **N°** | **Rt** (min) | **Organic acid** | **EPV** (mg/g) |
|---|---|---|---|
| 1 | 2.94 | Oxalic acid | 52.47±0.00 |
| 2 | 3.15 | Malic acid | 117.79±0.01 |
| 3 | 3.34 | Ascorbic acid | 28.06±0.00 |
| 4 | 3.69 | Citric acid | 42.85±0.00 |
| 5 | 3.94 | Succinic acid | 28.36±0.01 |
| 6 | 4.38 | Fumaric acid | 2.62±0.00 |
| | | ***Total*** | ***272.16*±0.01** |

### 2.2 Larval toxicity of EPV fractions

Analysis of the results made it possible to determine the 50% lethal concentration ($LC_{50}$) of EPV ($LC_{50}$=1.615 mg/mL). Referring to Mousseux's table, this value is greater than 0.1 mg/mL. According to Mousseux's scale, the EPV fraction is considered to be practically non-toxic to *Artemia salina* larvae.

### 2.3 Acute oral toxicity

All mice survived during the acute oral toxicity study of EPV. The observational phase reported no clinical signs of toxicity or mortality during the study period. In addition, no suspicious or unusual ethological manifestations were observed during the 14-day period.

*2.3.1 Body weight variation*

No significant weight variation was observed during the acute oral toxicity study. The study animals did not lose weight during the study period. Statistical analysis showed a statistically significant weight gain in individuals receiving EPV compared to the NaCl control (Figure 3).

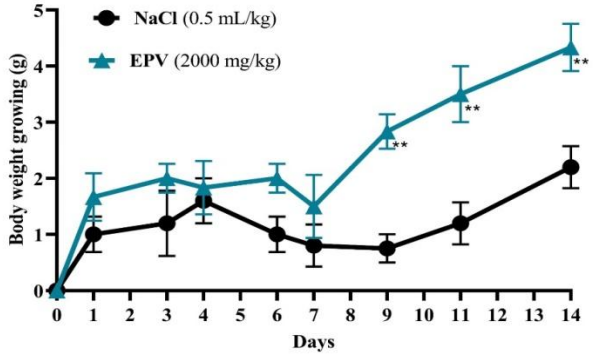



Figure 3: Weight change curve for mice during the acute oral toxicity study of EPV (n=5)

*Abbreviations: EPV: Ethyl acetate fraction of P. vulgaris.* Note :**= p<0.01 (EPV vs NaCl)

*2.3.2 Water and food consumption of mice in the study*

Figures 4-A and 4-B show the water and food consumption of the mice during the 14 days of the study. Statistical analysis did not reveal any significant differences in the diet and water intake of the mice in the study.

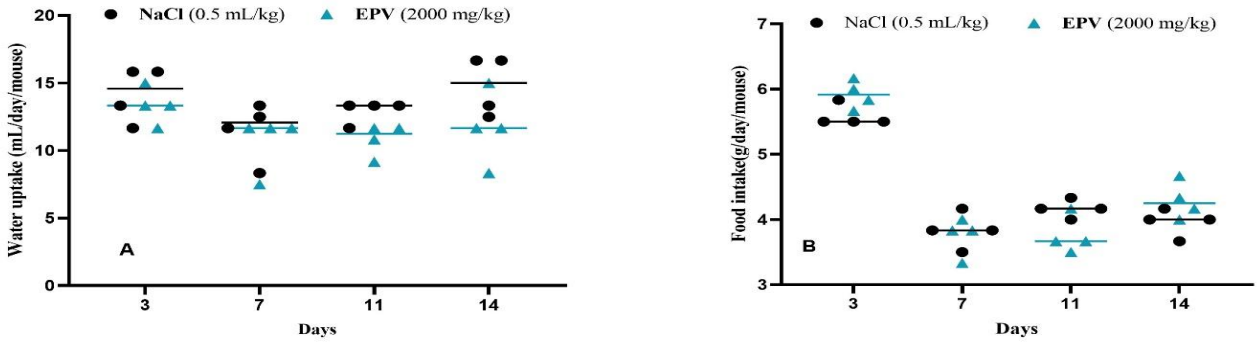



Figure 4: Histogram of water (A) and food (B) consumption by mice during the study.

*Abbreviations: EPV: Ethyl acetate fraction of P. vulgaris*

*2.3.3 Weight of vital organs*

The relative weights of vital organs such as the liver, heart, lungs, spleen, and kidneys are shown in Table II. Analysis of the data shows that there are no significant differences in relative weight between the organs of the control group and those of the treated group. Furthermore, no morphological alterations in size, appearance, or color were observed during the autopsy.

Table II: Average relative weight of vital organs (g/100 g body weight)

| Testing substances | Liver | Heart | Lung | Spleen | Kindney |
|---|---|---|---|---|---|
| **NaCl** *(0.5 mL/kg)* | 4.91±0.93 | 0.47±0.80 | 0.83±0.15 | 0.41±0.09 | 1.14±0.22 |
| **EPV** *(2000 mg/kg)* | 4.08±0.23 | 0.43±0.03 | 0.65±0.07 | 0.33±0.04 | 0.98±0.12 |

*Abbreviation:* ***EPV****: acetate ethyl fraction of P. vulgaris*

### 2.4 Diuretic effects of the ethyl acetate fraction of *Phaseolus vulgaris*

*2.4.1 Effect of EPV on urine excretion*

During the study, all animals excreted a volume of urine that depended on the substance or treatment received. Analysis of urine volumes showed an increase in the amount of urine in the groups treated with the reference substances furosemide, amiloride, and hydrochlorothiazide (P<0.05) throughout the study (figure 5). Similarly, rats receiving the EPV fraction (10 and 20 mg/kg) produced a volume of urine greater than that of the control group (P<0.05). hydrochlorothiazide (P<0.05) throughout the study. Similarly, rats receiving the EPV fraction (10 and 20 mg/kg) produced a higher urine volume than the control group. From the third hour onwards, the individuals in the EPV (10 mg/kg) and EPV (20 mg/kg) groups produced 4.17 times and 3.09 times the volume of urine collected from the rats in the negative control group, respectively. These differences were statistically significant for both categories (control and treatment) compared to the control (P<0.05). From the sixth hour onwards, the volumes collected from rats fed EPV 10 mg/kg and 20 mg/kg were 3.97 times and 3.07 times greater than those from the negative controls, respectively. After 24 hours, the urine volume of rats receiving a single dose of 10 and 20 mg/kg compared to the urine volume excreted by the controls was 1.45 and 1.17, respectively. Thus, the 20 mg/kg dose of EPV did not show a significant effect at this time.

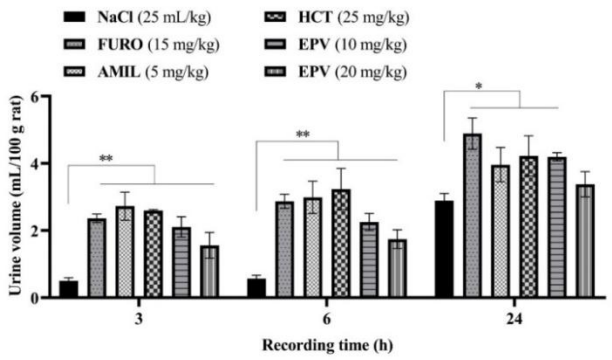



Figure 5: Effect of EPV on urinary volume during the study (n=5).

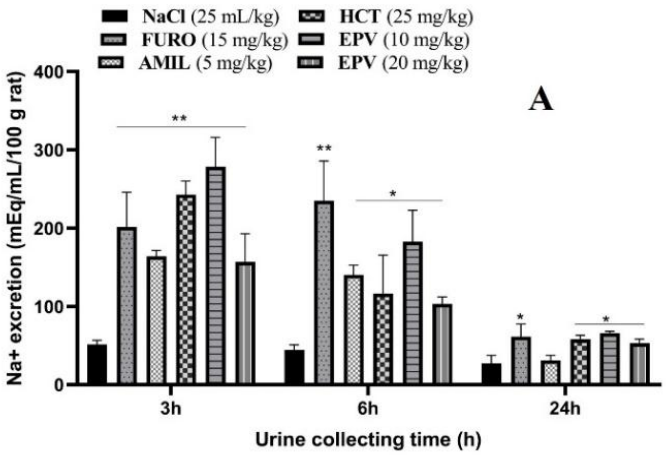
Abbreviation: **EPV**: acetate ethyl fraction of *P. vulgaris*; **FURO**: Furosemide; **AMIL**: Amiloride; **HCT**: Hydrochlorothiazide, Note: **=p<0.01; *=p<0.05

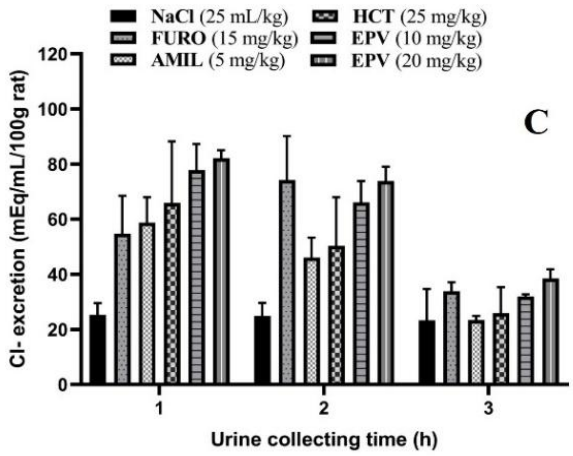
*2.4.2 Effect of EPV on electrolyte excretion*

Figure 6-A shows the urinary sodium ion concentrations for each group treated during the study of the diuretic effects of EPV. The rats treated with EPV 10, and 20 mg/kg had natriuria of 4.31times, 2.78 times higher in three hours than the control, 3.58 times, 2.09 times higher in six hours than the control, 2.23 times,1.93 times higher in the 24-hour natriuria of the rats receiving distilled water. In EPV extractions of 10mg/kg and 20 mg/kg, the value of natriuresis was statistically significant compared to control (distilled water). A similar observation was reported in the groups treated with furosemide and hydrochlorothiazide. Only rats fed amiloride recorded natriuria similar to the control (p>0.05).

The histograms in figure 6-B represent the urinary potassium ion concentrations of the rats in the study. In the third and sixth hours of the study, the electrolyte analyses of urine excreted by treated rats showed statistically higher potassium concentrations than in the control group except in the amiloride-fed group (P<0.05). The analysis report showed that the excretion of potassium ions in the 3rd-hour control was2.26 times higher and 4.46 times higher in EPV 10 mg/kg and 20 mg/kg. Subsequently, the excretion rate was 2.08 and 2.48hours in 6 hours,1.37 and 1.65 hours in 24 hours. Thus, it was reported that after 24 hours, only the furosemide and EPV (20 mg/kg) group had significantly higher kaliuria than the control group.

The concentration of chlorides in urine collected during the study is illustrated by the histograms in the figure 6-C. After six hours, the amounts of chloride produced in all rats receiving reference substances and EPV fractions were statistically higher than those in control groups (p<0.05). In fact, chloride concentrations in urine from rats fed 10 and 20 mg/kg EPV were 4.31 times higher than the control group at 3 hours and 2.79 times higher than the control group at 6 hours, respectively. At 24 hours, no statistical difference was observed between the chloride concentrations excreted by the control group and the other groups.



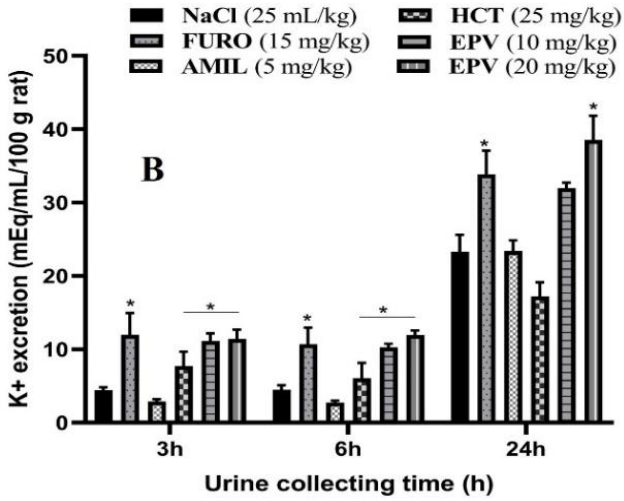




Figure 6: Effect of EPV on urinary sodium (A), potassium (B) and chloride (C) concentration during the study (n=5).

Abbreviation: **EPV**: acetate ethyl fraction of *P. vulgaris*; **FURO**: Furosemide; **AMIL**: Amiloride; **HCT**: Hydrochlorothiazide, Note: **=p<0.01; *=p<0.05

*2.4.3 Effect of EPV on urine pH*

During the study of the diuretic effect of EPV, the pH of urine collected at the 3rd hour was 7.29±0.13, 6.40±0.19, and 7.55±0.00 for the control, furosemide, amiloride, and hydrochlorothiazide, respectively. A slight change in pH was observed for the control, furosemide, amiloride, and hydrochlorothiazide 7.55±0.00 and 6.38±0.34 for the control, furosemide, amiloride, and hydrochlorothiazide, respectively. A slight change in these figures was observed at the 6-hour mark, but this did not significantly affect the acidity or alkalinity of the urine collected previously (P>0.05). At this collection time, the pH values were 6.98±0.22, 6.41±0.24, 7.55±0.25, and 6.82±0.30 for rats receiving distilled water, furosemide, amiloride, and hydrochlorothiazide, respectively. Subsequently, measurements of the pH of 24-hour urine showed no statistical differences between the control and the pharmacological references. In rats treated with EPV, urine collected at the 3-hour mark showed a significant difference in acidity, with pH values of 5.77±0.06 and 6.56±0.16 for EPV doses of 10 mg/kg and 20 mg/kg, respectively (P<0.05). Similarly, urine from rats treated with EPV maintained its acidity until the 6th hour, with intensities of 5.80±0.12 and 6.47±0.21 for EPV 10 mg/kg and 20 mg/kg, respectively (P<0.05). The urinary pH values recorded in these two groups of rats improved to 7.07±0.05 and 7.15±0.36, respectively, for doses of 10 mg/kg and 20 mg/kg of EPV after 24 hours. Thus, statistical analysis of 24-hour urine samples showed no significant difference between the groups treated with EPV and the rats given distilled water (P>0.05).

*2.4.3 Impact of EPV diuretic indices*

The calculation of natriuretic indices ([Na+]/[K+]) and saliuretic indices ([Cl-]/[Na+] +K+]) are presented in the table. Analysis of the table showed natriuretic indices greater than 1 for furosemide, hydrochlorothiazide, and EPV 10 mg/kg during the study. Rats fed amiloride reported a natriuretic index lower than 1. For the 20 mg/kg dose, the natriuretic index increased, with values below 1 (0.69 at 3 hours and 0.84 at 6 hours) and then above 1 (1.17) at 24 hours.

For the $[Cl^-]/[Na^+]+[K^+]$ index, values were generally below 0.8 for furosemide, hydrochlorothiazide, and 10 and 20 mg/kg doses of EPV. In contrast, amiloride induced $[Cl^-]/[Na^+]+[K^+]$ indices greater than 1.2 at the 3rd hour, indicating excessive loss during this period in the rats in the study. Subsequently, this chloride ion loss was gradually reduced without, however, reaching the normal electrolyte equilibrium value (index=1).

Table III: Impact of ethyl acetate fraction of *P. vulgaris* on diuretic indicator

| Substances | $[Na^+]/[K^+]$ | | | $[Cl^-]/[Na^+]+K^+]$ | | |
|---|---|---|---|---|---|---|
| | 3h | 6h | 24h | 3h | 6h | 24h |
| **NaCl** (25 mL/kg) | - | - | - | - | - | - |
| **Furosemide** (5 mg/kg) | 1.48 | 2.25 | 1.55 | 0.57 | 0.45 | 0.39 |
| **Amiloride** (5 mg/kg) | 0.70 | 0.57 | 0.45 | 1.95 | 1.18 | 1.20 |
| **Hydrochlorothiazide** (25 mg/kg) | 4.78 | 5.41 | 1.67 | 0.26 | 0.27 | 0.37 |
| **EPV** (10 mg/kg) | 1.90 | 1.72 | 1.63 | 0.47 | 0.47 | 0.38 |
| **EPV** (20 mg/kg) | 0.63 | 0.84 | 1.17 | 0.64 | 0.65 | 0.46 |

## 3. DISCUSSION

Herbal products have long been considered pillars in the development of therapeutic tools due to the bioactive compounds they contain. Diuretics, used for their ability to increase urine volume and/or electrolytes, are a family of drugs highly recommended for the management of high blood pressure, renal dysfunction, edema, and uncontrolled hypervolemia (1, 5). The corrective action of these diuretics helps inhibit water reabsorption and increase electrolyte excretion. In this study, the urinary volumes and electrolyte concentrations of randomized rats were measured to assess the diuretic activity of the EPV fraction. In addition, the pH of the collected urine was determined to evaluate the acid-base electrolyte balance at the renal level. Thus, similar to pharmacological references such as furosemide, amiloride, and hydrochlorothiazide, the EPV fraction at the doses tested caused an increase in volume and an acceleration of urinary flow in rats at each time of urinary volume recording. This dose-dependent diuretic action of EPV showed a profile relatively similar to that of furosemide in terms of urinary flow, with urinary excretion being higher during the first six hours. Similar studies have also reported dose-dependent diuretic effects of plant extracts (20–23). The mechanism of action by which EPV induces its diuretic effect cannot be elucidated through this study. Further studies will be conducted to determine the likely targets and pharmacological actions. For now, it is well known that furosemide, a diuretic drug, is used clinically for its rapid diuretic action in isolated cases of pronounced hypervolemia and especially in the chronic treatment of hypertensive patients with renal dysfunction or a history of renal disease. The electrolyte balance of the urine of rats treated with EPV, as measured by urinary ionogram, showed a natriuretic-type diuretic action. This is manifested by effective natriuretic effects that mimic, in some areas, the potassium-sparing effects of furosemide and hydrochlorothiazide. These two diuretic drugs act preferentially on the ascending limb of Henle's loop, inhibiting the active reabsorption of chloride ions while increasing the excretion of $Na^+$ and $K^+$ ions (19, 24). This reduction in potassium caused by furosemide and EPV can lead to hypokalemia; hence, the recommendation to use potassium-sparing diuretics in chronic treatment in clinical practice. The present study did not report any similarity between the diuretic action of EPV and that of amiloride. Amiloride, a potassium-sparing diuretic, is widely used for its ability to inhibit mainly the ENaC sodium channel, thereby reducing $Na^+$ absorption and increasing $K^+$ excretion (25, 26). Although the precise mechanism of action of EPV has not been determined, it is possible that this EPV fraction acts like furosemide in the loop of Henle and the distal convoluted tubule while promoting diuretic activity by inhibiting water reabsorption associated with the excretion of sodium and potassium cations (27). The diversity and high organic acid content of EPV could also contribute to the transport and delivery of bioactive compounds to the site of action. It has been reported that furosemide owes its rapid diuretic action to an organic acid pump that facilitates it reaching its target (28). The variation in pH from acidic to neutral in the urine produced by rats fed EPV could be explained by the presence of organic acid in this substance. Indeed, the metabolism of organic acids contributes to urine acidification. Specifically, citric, malic, and succinic acids can modulate urinary pH and renal tubular function, thereby promoting the excretion of water and $Na^+$. Thus, they cause a decrease in blood pressure by reducing blood volume. Authors have shown that extracts of *Halosarcia indica, S. septemtrionalis*, and *Cola nitida*, rich in malic, caffeic, ferulic, and chlorogenic acids, contribute to increased diuresis while decreasing urinary pH (29, 30). This study showed interesting levels of organic acids such as citric, malic, fumaric, ascorbic, and succinic acids in EPV. Authors have reported not only antihypertensive and antioxidant effects but also diuretic effects of these phenolic acids (31, 32). Recent work has shown the antihypertensive, vasodilator, and antioxidant properties of the ethyl acetate fraction of *Phaseolus vulgaris* (12). In addition to their use in nutritional health to balance the diet, these unsaturated fatty acids play an essential role in pH regulation and the optimal transport of drugs to their site of action (33, 34).

However, the safety of diuretics and the side effects directly or indirectly related to their use are widely reported (7, 35). In this study, the assessment of larval cytotoxicity and acute oral toxicity made it possible to determine tolerable doses without notable side effects. Although not all parameters involved in electrolyte balance were measured during the study, the absence of mortality and suspicious toxicity behaviors provides reassurance regarding the use of EPV for the treatment of hypertension. However, the lack of more detailed information on the architecture of the nephron, the vessels and cells involved in the glomerular filtration process, and the performance of renal function are limitations of the study.

## CONCLUSION:

The presence of different compounds with diverse pharmacological properties within the same extract would be a considerable asset for synergistic action. The richness of EPV in citric, malic, ascorbic, succinic, and fumaric acids would contribute to its diuretic and blood pressure-regulating effect through synergistic action with bioactive compounds such as chlorogenic acid, quercetin glycoside derivatives, and gallic acid. The diuretic effects demonstrated in this study support the antihypertensive properties of Phaseolus vulgaris and justify its use as a traditional treatment for high blood pressure.

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