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Study Of The Normoglycemic Activity Of Aqueous And Hydroethanolic Extracts Of The Leaves Of Pavetta Corymbosa (DC) F.M. Williams (Rubiaceae).

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Abstract
As part of efforts to promote traditional medicine, a pharmacological study was conducted on aqueous and hydroethanolic extracts of the leaves of Pavetta corymbosa, a plant used in traditional medicine. The study focused on evaluating the normoglycemic potential of these two types of extracts. In this evaluation, hyperglycemia was induced orally by administering glucose to rats at a dose of 0.5 g/mL (5 g/kg body weight) treated with aqueous and hydroethanolic extracts of Pavetta corymbosa leaves at a dose of 200 mg/kg body weight and metformin (the reference normoglycemic drug) at 100 mg/kg body weight. Regarding the aqueous extract, blood glucose levels decreased from 1.33 ± 0.13 g/L to 1.01 ± 0.28 g/L from the first hour to the twelfth hour after treatment. As for the hydroethanolic extract, blood glucose levels decreased from 1.33 ± 0.11 g/L to 0.63 ± 0.11 g/L from the first hour to the twelfth hour after treatment. This study showed that the hydroethanolic extract and metformin significantly reduced ($p < 0.05$) hyperglycemia in rats compared to the aqueous extract. This efficacy of the hydroethanolic extract could be explained by the presence of saponins and quinones in this extract compared to the aqueous extract. The efficacy of the hydroethanolic extract of Pavetta corymbosa leaves, similar to reference drugs for the treatment of hyperglycemia, could be a source of optimism for the treatment of diabetes in traditional medicine.

Keywords: *Aqueous extract, hydroethanolic extract, Pavetta corymbosa, normoglycemic.*

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I. Introduction

Traditional medicine, which uses medicinal plants to treat or prevent certain illnesses, is an ancient practice widely practiced in most parts of the world. Plants constitute a major source of medicines because they contain numerous bioactive molecules, most of which likely play a role in defending or protecting the body **(Usha *et al* ., 2014).** This traditional medicine is less expensive and accessible to all. It is with this perspective in mind that the plant species *Pavetta corymbosa*, from the Rubiaceae family, was used in this study. Its leaves are used in decoctions and infusions for the treatment of diabetes, malaria, fever, pain, inflammation, skin infections, and arthritis **(Adjanohoun *et al.,* 1979).** Furthermore, triphytochemistry has revealed the presence of chemical compounds in the aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves that possess pharmacological potential **(Miezan *et al.,* 2024).** The predominant active compounds are flavonoids, polyphenols, polyterpenes, and sterols. However, in addition to the compounds mentioned above, the hydroethanolic extract contains saponins and quinones **(Miezan *et al.,* 2024).** Furthermore, the Globally Harmonized System of Chemicals (GHS) considers aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves to be non-toxic substances **(Hodge and Sterner, 1943).** On the other hand, despite the widespread use of these leaves, no scientific studies have been conducted on their effectiveness in treating hyperglycemia. Therefore, the objective of this study is to evaluate the normoglycemic potential of the leaves of this plant, which is commonly used in traditional medicine.

II. Material And Methods

Material

Plant Material

For the evaluation of the normoglycemic potential of *Pavetta corymbosa*, the plant material consisted of leaves harvested in the Aboisso region (Côte d'Ivoire).

Animal Material

Albino rats (Rattus norvegicus) of the Wistar variety, weighing between 115 and 119 grams, were used in this study. These animals came from the animal facility of the Faculty of Biological and Pharmaceutical

Sciences at Félix Houphouët Boigny University (Côte d'Ivoire). The rats were kept in favorable breeding conditions, respecting the standards and good practices for laboratory animals. They were fed a standard complete pelleted diet. The animals received continuous access to tap water via water bottles.

Methods

Sampling

For this study, the plant material consisted of 3 kg of *Pavetta corymbosa* leaves harvested from the same site (Aboisso). This site was chosen for harvesting the plant material due to its accessibility and the abundance of *Pavetta corymbosa* there. The leaves were packed in biodegradable bags and transported in a van immediately after harvesting. The harvested leaves were dried in the dark in the laboratory for three weeks before being ground using a mechanical grinder (IKAMAG, Japan).

Preparation of the Aqueous Extract of *Pavetta corymbosa*

The aqueous extract was prepared by steeping 100 grams of *Pavetta corymbosa* leaf powder in 1 L of boiling distilled water for ten minutes. The resulting solution was filtered through cotton, then under vacuum using a Whatman paper filter. The filtrate was oven-dried at 40°C, yielding the total crude aqueous extract of *Pavetta corymbosa*.

Preparation of the Hydroethanolic Extract of *Pavetta corymbosa*

For the 70% hydroethanolic extract, the **Guédé-Guina** method **(1993)** was used. 100 g of *Pavetta corymbosa* leaf powder were used for this purpose. The resulting mixture was homogenized using a magnetic stirrer for one (1) day. The solution was filtered through cotton and then under vacuum under the same conditions as before. The resulting filtrate was concentrated using a rotary evaporator and then dried in an oven at 40°C. The powder obtained constituted the hydroethanolic extract of *Pavetta corymbasa*.

Normoglycemic Activity

The normoglycemic potential test was performed according to **Patrice's method (2017).** Hyperglycemia was induced by oral administration of glucose to rats at a dose of 0.5 g/mL, or 5 g/kg body weight. In this study, 24 rats were divided into four groups of six.

-Group 1 received 5 g/kg of glucose + 10 mL of an aqueous extract of *Pavetta corymbosa* leaves at 200 mg/kg body weight orally.

-Group 2 received 5 g/kg of glucose + 10 mL of a hydroethanolic extract of *Pavetta corymbosa* leaves at 200 mg/kg body weight orally.

-Group 3 received 5 g/kg of glucose + 10 mL of metformin (the reference glycemic control drug) at 100 mg/kg orally.

-Group 4 received 5 g/kg of glucose + 10 ml of distilled water orally.

The normal blood glucose level in each group of rats was between 0.63 and 1.15 g/L immediately before administration of the substances or distilled water at T_0. It was measured at 1, 2, 3, 5, 8, and 12 hours after administration using a glucometer.

Statistical Analysis

Results were expressed as mean ± standard deviation (SD) of the mean (mean ± SD). Data visualization and analysis were performed using Graph Pad Prism 5.0 software (Microsoft USA). Differences between means were determined using Dunnett's test: $p < 0.01$ (highly significant difference) and $p < 0.05$ (significant difference).

III. Results And Discussion

Results

Effect of aqueous and hydroethanolic extracts and metformin on induced hyperglycemia in rats

Before the study, the baseline blood glucose level in each group of rats was 0.60 ± 0.13 g/L (**Figure 1**).

One hour after treatment, the blood glucose levels in rats treated with aqueous and hydroethanolic extracts at a dose of 200 mg/kg, metformin (100 mg/kg), and distilled water increased. They increased from 0.60 ± 0.13 g/L to 1.33 ± 0.11 g/L with the hydroethanolic extract; and from 0.60 ± 0.13 g/L to 1.29 ± 0.15 g/L with metformin. The increases in blood glucose levels were 0.60 ± 0.13 g/L to 1.33 ± 0.13 g/L with the aqueous extract and 0.60 ± 0.13 g/L to 1.50 g/L with distilled water. This represents a respective increase of 121.56% (hydroethanolic extract), 115% (metformin), 121.56% (aqueous extract), and 150% for distilled water (**Figure 2**).

Two hours after treatment, the 200 mg/kg dose of hydroethanolic extract and metformin (100 mg/kg) resulted in a significant decrease in blood glucose ($p < 0.05$) compared to the aqueous extract (200 mg/kg), but a more significant decrease compared to distilled water ($p < 0.01$). This decrease in blood glucose levels ranged

from 1.33 ± 0.11 g/L to 1.08 ± 0.18 g/L (hydroethanolic extract), from 1.29 ± 0.15 g/L to 1.07 ± 0.10 g/L (metformin), from 1.33 ± 0.13 g/L to 1.30 ± 0.17 g/L (aqueous extract), and from 1.50 ± 0.17 g/L (distilled water). This represents a decrease in blood glucose levels of 23.18% for the hydroethanolic extract, 20.56% for metformin, 12.71% for the aqueous extract, and 5.33% for distilled water. The hydroethanolic extract and metformin significantly reduced blood glucose levels compared to the aqueous extract ($p < 0.05$) and very significantly reduced blood glucose levels compared to distilled water ($p < 0.01$). There was no significant difference between the hydroethanolic extract and metformin ($p > 0.01$). However, there was a significant difference between the aqueous extract and distilled water ($p < 0.05$; **Figure 3**).

Furthermore, between the third and fifth hours, between the fifth and eighth hours, and between the eighth and twelfth hours, a reduction in blood glucose levels was observed. During the periods described above, the decrease in blood glucose levels in rats was much more pronounced with the hydroethanolic extract and metformin compared to the aqueous extract ($p < 0.05$). The aqueous extract showed a more pronounced difference compared to distilled water ($p < 0.01$). However, there was no significant difference between the hydroethanolic extract and metformin ($p > 0.01$) between these time points. In contrast, there was a significant difference between the aqueous extract and distilled water ($p < 0.01$).

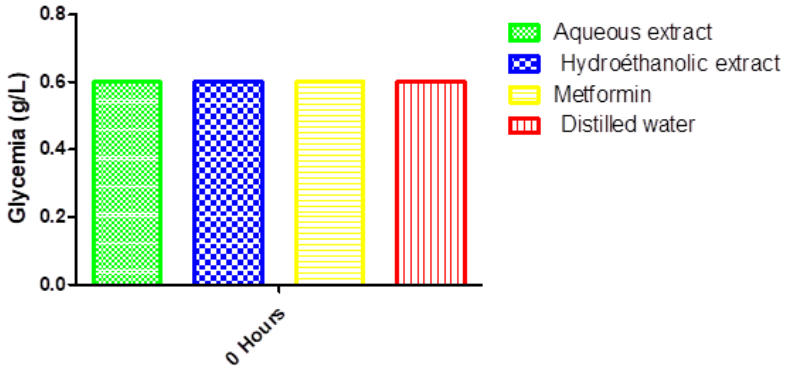

Figure 1: Initial glycemia of rats

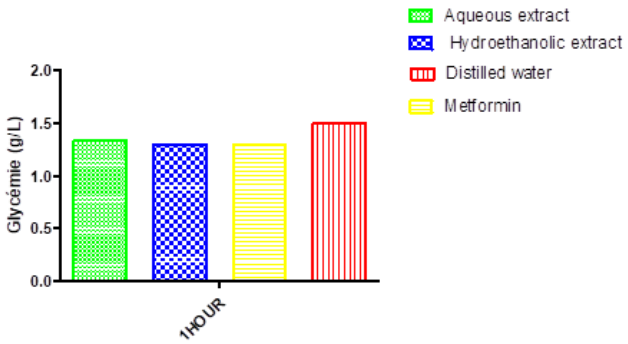

Figure 2: effect of aqueous and hydroethanolic extracts of Pavetta corymbosa leaves and metformin on blood glucose levels in the first hour

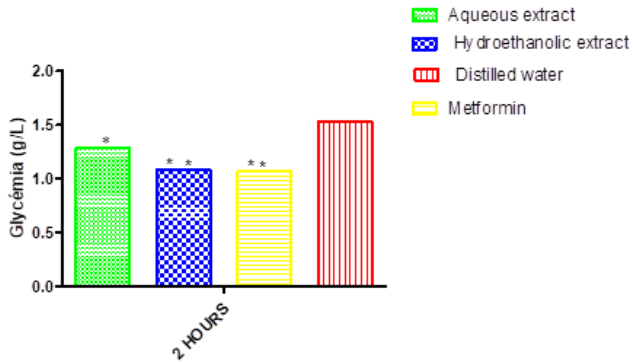

Figure 3: Effect of aqueous and hydroethanolic extracts of Pavetta corymbosa leaves and metformin on blood glucose at the second hour

Finally, at the twelfth hour, the blood glucose levels of rats treated with the hydroethanolic extract and metformin decreased from 0.69 ± 0.15 g/L to 0.63 ± 0.11 g/L and from 0.66 ± 0.17 g/L to 0.60 ± 0.20 g/L, respectively, representing a decrease in blood glucose of 9.52% and 10%. As for the aqueous extract and distilled water, the blood glucose levels of rats treated with these two substances were reduced from 1.01 ± 0.28 g/L to 0.99 ± 0.38 g/L and from 1.09 ± 0.33 g/L to 1.085 ± 0.37 g/L, respectively. Blood glucose levels decreased by 2% and 0.46%, respectively. The return to the initial blood glucose level occurs at the twelfth hour, either 0.60 ± 0.20 g/L for metformin and 0.63 ± 0.11 g/L for the hydroethanolic extract (**figure 4**).

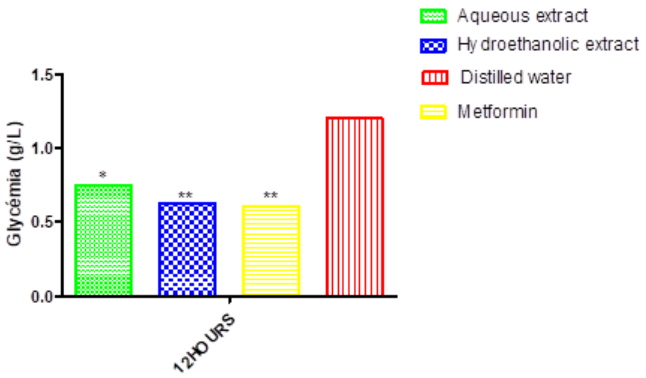

Figure 4: Effect of aqueous and hydroethanolic extracts of Pavetta corymbosa leaves and metformin on blood glucose at the twelfth hour

Normoglycemic Potential of Aqueous and Hydroethanolic Extracts of *Pavetta corymbosa* Leaves

Oral administration of glucose at a dose of 5 g/kg bw induced hyperglycemia in rats treated with the different aqueous (200 mg/kg bw) and hydroethanolic (200 mg/kg bw) extracts of *Pavetta corymbosa* leaves and metformin (100 mg/kg bw) within the first hour. Peak blood glucose levels were 1.33 ± 0.22 g/L, 1.30 ± 0.37 g/L, and 1.29 ± 0.33 g/L for the aqueous, hydroethanolic, and metformin extracts, respectively. In contrast, the peak hyperglycemia in rats treated with distilled water was 1.55 ± 0.36 g/L within the second hour. Subsequently, this hyperglycemia gradually decreased over the second hour. The blood glucose levels of rats receiving only distilled water decreased very slowly over time (**Figure 5**). In contrast, the administration of metformin (100 mg/kg body weight) and the hydroethanolic extract (200 mg/kg body weight) resulted in a reduction of glucose-induced blood glucose levels over time (**Figure 5**). A return to baseline blood glucose levels occurred at the twelfth hour, at 0.60 ± 0.20 g/L for metformin and 0.63 ± 0.11 g/L for the hydroethanolic extract. Metformin (100 mg/kg bw) and hydroethanolic extract (200 mg/kg bw) of *Pavetta corymbosa* leaves, administered 1 hour after glucose administration, resulted in a greater reduction in induced hyperglycemia over time compared to the aqueous extract (200 mg/kg bw) with P< 0.05. A return to the initial blood glucose levels of the rats was observed at the twelfth hour.

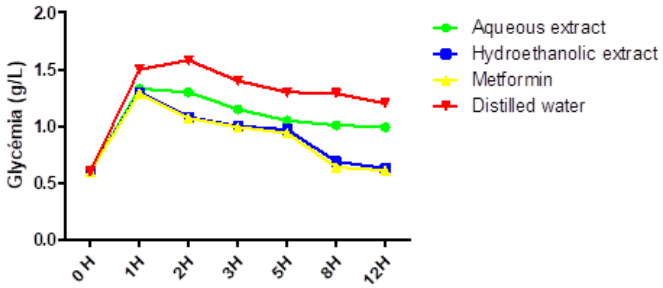

Figure 5 : Dose-response effect of Pavetta corymbosa leaf extracts and metformin on blood glucose over 12 hours

Discussion

Administering the glucose solution to rats treated with metformin (100 mg/kg) and the aqueous and hydroethanolic extracts at a dose of 200 mg/kg bw resulted in a significant decrease in blood glucose levels during the study compared to rats receiving only distilled water. Indeed, substances such as polyphenols and flavonoids contained in both types of extracts **(Patrice *et al.*, 2024)** are generally recognized as having blood glucose-lowering effects **(Mangambu *et al.*, 2014).** The similar effects of the extracts on blood glucose to those of

metformin suggest that the extracts may act through the same mechanism as the reference normo-hyperglycemic substance used. The decrease in blood glucose levels is due to the action of the extracts, as well as the drug, through their effects on stimulating pancreatic β cells to secrete insulin **(Zheng *et al.,* 2012)** or facilitating glucose transport to cells or inhibiting intestinal glucose transporters (10). These authors reported that the antihyperglycemic action of these substances could be explained by improved insulin sensitivity. This result is consistent with the work of Kouamé et al. **(Kouame *et al.,* 2017),** who showed that at a dose of 200 mg/kg bw, aqueous and hydroethanolic extracts of *Xylopia villosa* bark significantly reduced hyperglycemia. However, if the effect of the hydroethanolic extract is greater than that of the aqueous extract, this could be explained by the presence of saponins and quinones in said extract **(Patrice *et al*., 2024)**. This finding is consistent with the work of Stalin et al. (**Stalin *et al.,* 2012**), who highlighted that oral administration of 200 mg/kg bw of hydroethanolic extract of *Ficus carica* L. leaves significantly reduced hyperglycemia in Wistar rats compared to the aqueous extract at the same dose. The blood glucose-lowering effect of the hydroethanolic extract at a dose of 200 mg/kg body weight could support the use of *Pavetta corymbosa* leaves in normalizing blood glucose levels and even in the management of diabetes.

IV. Conclusion

The results obtained in this study showed that both the aqueous and hydroethanolic extracts of Pavetta corymbosa leaves possess hepatoprotective and blood glucose-lowering properties. However, the hydroethanolic extract demonstrated greater activity than the aqueous extract. These results provide a scientific basis that justifies the traditional use of *Pavetta corymbosa* leaves in the management of various diseases.

Acknowledgment

The authors thank Mr. Ouoplé Clément (UFR of Pharmaceutical and Biological Sciences, Félix Houphouët Boigny University) for his help with the experiments.

Ethics Approval

The experimental procedures and protocols used in this study were approved by the ethics committee, Health Sciences Committee, Félix Houphouët-Boigny University. These guidelines were in accordance with those of the European Council Legislation 87/607/EEC for the protection of experimental animals. Every effort has been made to minimize animal suffering and reduce the number of animals used.

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