ejpmr, 2019,6(9), 559-564

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF THE HYPOGLYCEMIC AND HEPATOPROTECTIVE ACTIVITY OF THE AQUEOUS TOTAL EXTRACT OF *PARQUETINA NIGRESCENS* (APOCYNACEAE) IN RATS

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Article Received on 23/07/2019 **Article Revised on 13/08/2019** **Article Accepted on 03/09/2019**

SUMMARY
Parquetina nigrescens (Asclepiadacee) is a plant of Ivorian medicinal flora. It is traditionally used in the treatment of diabetes, high blood pressure, diarrhea, and anemia, etc. The objective of this study is to evaluate the hypoglycemic and hepatoprotective effects of the total aqueous extract (ETAq) of this plant.

KEYWORDS: Bilirubin, glyceamia, transaminases, hepatocytes.

INTRODUCTION

Traditional medicine uses many herbal recipes for the treatment of diabetes and liver disease (Aderibigbe, 2011). Among these plants, Parquetina nigrescens (Asclepiadaceae) has a prominent place **(**Burkill, 1997). Parquetina nigrescens occurs in much of Africa, from Senegal to Sudan and south to Zambia, Angola and eastern Zimbabwe, through central and eastern Africa. It is a Lianescente plant, up to 8 m long, glabrous, with copious latex. These leaves are opposite, simple and whole, almost sessile. Latex is very toxic and commonly used as an ingredient in arrow poisons, particularly in Central Africa (Burkill, 1997). The fibers of the inner bark are soft, white and strong, and are commonly used to make fishing nets and fishing lines of excellent quality. The stems are also used as ropes. The latex is white, black when it hardens and it has been exploited in R.D. Congo in the past to give black rubber. The flowers are quite showy and have an ornamental potential. Latex and leaf sap cause a burning sensation on the skin and are applied externally to tumors, abscesses, wounds and burns (Aderibigbe, 2011); they also darken the scars and are applied to the thorns planted in the skin to extract them. The leaves are applied as plaster on the wounds; the crushed leaves are applied to the cutaneous affections and against the lice. The maceration of leaves is applied to the legs of the rachitic children, on the head to treat headache and on the flanks to treat the pains alongside. (Imaga *et al* 2010). The powdered bark is applied to incisions in the skin to treat rheumatism. The juice of the leaves, without latex, is used as eye drops to treat conjunctivitis and jaundice. Latex is known to cause blindness (Neuwinger, 2000).

Our work, which is a contribution to the valorization of the African pharmacopoeia in general and Ivoirian in particular, aims to evaluate the hypoglycemic and hepatoprotective activity of the total aqueous extract (ETAq) of the leaf powder of Parquetina nigrescens (Asclepiadaceae) in the anemic rat. Specifically, the effects of ETAq Parquetina nigrescens (Asclepiadaceae) on the variation of blood glucose or glucose levels, and on the change in biochemical parameters such as ASAT and ALAT transaminases, will be studied. Lactic-dehydrogenase (LDH) and Bilirubin.

I. MATERIAL AND METHODS

The effects of different doses of *Parquetina nigrescens* aqueous total extract (ETAq) on serum glucose variation, liver markers, and bilirubin were studied for 28 days in rats. Glucose and transaminases were assayed and analyzed as was bilirubin. Hepatocyte protection was assessed by the no significant change in the percentage of intracellular enzymes (LDH, ALT and AST) and bilirubin relative to those of the control.

1. MATERIAL

1.1. Plant material

The plant biological material used consists of leaves of *Parquetina nigrescens*, harvested in Godjiboué, in a village of Sassandra (Central West of Ivory Coast), in June 2010. They were subsequently washed and cut into small pieces and dried out of the sun at room temperature

(25-30 ° C). Four (04) weeks after drying, these dried leaves were reduced to powder and stored in plastic bags. This powder was used to prepare the total aqueous extract.

1.2. Animal material

Rattus norvegicus rats of Wistar strain weighing between 200 and 300 g, aged from 1 to 2 months were used as animal material for this study. These animals, supplied by the Pasteur Institute of Ivory Coast (IPCI), were acclimatized for three weeks in the Biochemical Pharmacodynamics laboratory to harmonize their physiological state before any experimentation. They were nourished during the whole grain experiment provided by FACI.

2. METHODS

2.1. Preparation of the aqueous extract

The aqueous total extract of *Parquetina nigrescens* was prepared by dissolving one hundred grams (100g) of powder of this plant in one liter of distilled water and then homogenized for two (02) hours at room temperature 25ºC using a magnetic stirrer IKA MAG. The homogenate obtained was filtered twice on hydrophilic cotton and once on whatman 3 mm filter paper. The filtrate obtained was evaporated using the BÜCHI rotary evaporator at 60 ° C. We get a brown deposit at the bottom of the balloon. This deposit constituted the total aqueous extract of Parquetina nigrescens and was used to prepare the different concentrations and doses of the product (Guede-Guina *et al*, 1993, Zirihi *et al*., 2001).

2.2. Chemical Screening of the aqueous extract of parquetina nigrescens (ETAq)

2.2.1. Search for tannins

Two to three drops of FeCl 32% were added to 2 mL of 27 mg / mL leaf extract. After a few minutes, a blue-black color appears and a precipitate characteristic of the tannins (Karumi *et al*., 2004**)**.

2.2.2. Search for flavonoids

Five milliliters of extract was treated with a few drops of concentrated HCl. A small amount of magnesium turnings are introduced and allowed to react. The appearance of a red or orange color characterizes the presence of aglycone flavones (Karumi *et al*., 2004).

2.2.3. Search for alkaloids

To the residue obtained by evaporation of 25 ml of extract is added 5 ml of 2 NHCl, the whole is heated in a water bath. The mixture is filtered using a filter paper and tested with Mayer's reagent. The presence of alkaloids is characterized by the appearance of a turbidity or a white precipitate **(**Brunetton, 1999**)**.

2.2.4. Research of sterols and polyterpenes

0.5 g of extract are dissolved in 0.5 ml of chloroform. 0.5 ml of acetic anhydride and a few drops of fuming sulfuric acid are added. A change in color from purple to blue is characteristic of sterols or polyterpenes when this change is from purple to green **(**Bruneton, 1999**)**.

2.2.5. Determination of glyceamia and liver markers

2.2.5.1. Determination of blood glucose

The assay method used is the enzymatic method (Djedje, 2002). It consists in oxidizing glucose by the enzyme glucose oxidase with production of gluconic diacid and dihydrogen peroxide (H_2O_2).

2.2.5.2. Determination of lactate dehydrogenase (LDH)

LDH was assayed by the method of Henry et al., 1974

Pyruvate + NADPH + H^+ L-lactate + NAD^+

The decrease in absorbance due to the conversion of NADH to Andes directly related to the activity of LDH.

2.2.5.2. Determination of alanine aminotransferase (ALAT)

ALAT is dosed according to the method recommended by the International Federation of Clinical Chemistry (FICC) (Bergmeyer, 1980). The assay is based on the following principle: ALAT catalyzes the transfer of the amine group from alanine to α-ketoglutarate to form pyruvate and L-glutamate. Pyruvate is converted to lactate by lactate dehydrogenase. The rate of reduction of NADH is proportional to the amount of pyruvate formed in the medium and therefore to the activity of alanine. This activity is determined by measuring the absorbance at 350 nm due to the decrease of NADH in the serum.

2.2.5.4. Assay of aspartate aminotransferase (ASAT)

ASAT was assayed according to the method of Karmen (1955) as modified by Bergmeyer *et al*., (1978). ASAT transfers the amino group of aspartate to the carbon atom of α-keto glutarate with formation of glutamate and oxaloacetate. The latter is then reduced to malate by malate dehydrogenase (MDH) in the presence of reduced NADH which oxidizes to NAD^+. However, the catalytic activity of ASAT is obtained by determining the disappearance of NADH at 340 nm.

2.2.6. Determination of Bilirubin

Bilirubin was assayed in anemic rats by induction of phenylhydrazine hydrochloride from a blood sample, using a laboratory kit.

2.2.7. Statistical analysis

The statistical analysis of the values and the graphical representation of the data were carried out with Graph Pad Prism 5 software (Microsoft). The average value is accompanied by the standard error on the mean (mean ± SEM). The statistical analysis of the results was performed using the one-way analysis of variances (ANOVA) followed by the Tukey multiple comparison test. $P < 0.001$ is considered significant.

II. RESULTS AND DISCUSSION

1. Results

1.1. Phytochemical screening of the ETAq of *Parquetina nigrescens*

The results of the phytochemical screening show that the aqueous total extract (ETAq) of Parquetina nigrescens very abundantly contains alkaloids, abundantly flavonoids, then polyphenols, polyterpenes, tannins and sterols. (Table 1).

Table 1: Chemical Composition of The Aqueous Total Extract of *Parquetina Nigrescens.*

Chemical groups	% Presence
Alkaloids	+++
Flavonoids	++
Polyphenols	+
Polyterpenes	+
Tannins	+
Sterols	+

(+++) = very abundant; (++) = abundant; (+) = presence

1.2. Effect of ETAQ of *Parquetina nigrescens* on the variation of blood glucose

The results of figure 1 present the effects of different doses (0, 1000, 2000, 2500, 3000 and 4000 mg / kg.pc) of the total aqueous extract (ETAq) of *Parquetina nigrescens* on the evolution of blood glucose levels in normal rats. The control glucose level is 0.77 g / L.

The administration of the different doses of ETAq *Parquetina nigrescens* resulted in a significant decrease ($p < 0.001$) of blood glucose compared to rats in the control group. The blood glucose level changes from 0.77 g / L (control blood sugar) to 0.56 g / L and then to 0.53 g / l; 0.42 g / L; 0.31g / L and finally 0.15g / L when the rats are treated with doses ranging from 1000, 2000; 2500, 3000 and 4000 mg / kg pc respectively.

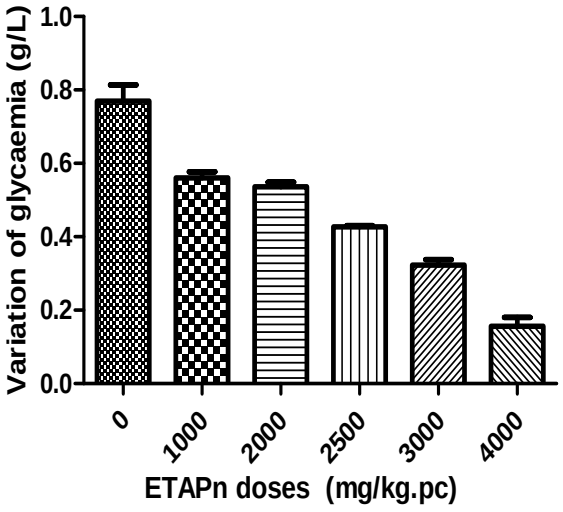

Figure 1: Effect of different doses of ETAq of *Parquetina nigrescens* on the variation of blood sugar levels in rats.

1.3. Effect of different doses of *Parquetina nigrescens* on the serum variation of the transaminase levels ASAT and ALAT

The influence of different doses of ETAq Parquetina nigrescens on the change in serum levels of transaminases ASAT and ALAT in rats is summarized in figures 2 and 3. *Parquetina nigrescens* ETAq, administered at increasing doses of 1000, 2000, 2500, 3000 and 4000 mg / kg bw to rats, did not cause a significant ($p > 0.05$) change in serum ASAT and ALAT transaminases relative to rats in the control group (A).

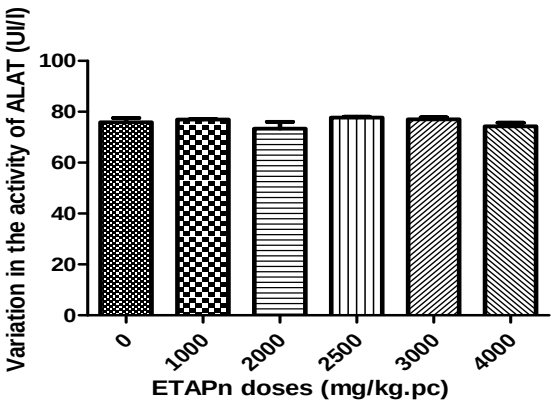

Figure 2: Effect of different doses of P. nigrescens on serum rate variation transaminases ALAT and ASAT.

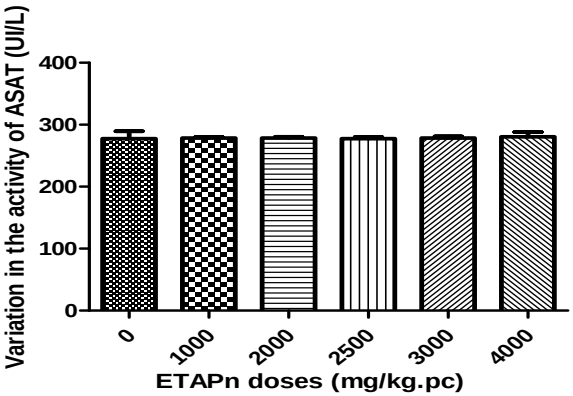

Figure 3: Effect of different doses of *Parquetina nigrescens* on serum rate variation transaminases ALAT and ASAT.

1.4. Effect of ETAq of *Parquetina nigrescens* on the variation of the serum LDH level

The histograms in figure 3 show the effects of different doses of *Parquetina nigrescens* ETAq on the change in serum Lactico-dehydrogenase (LDH). Compared to the animals in the control group, the different doses of *Parquetina nigrescens* did not have a significant effect on serum LDH variation.

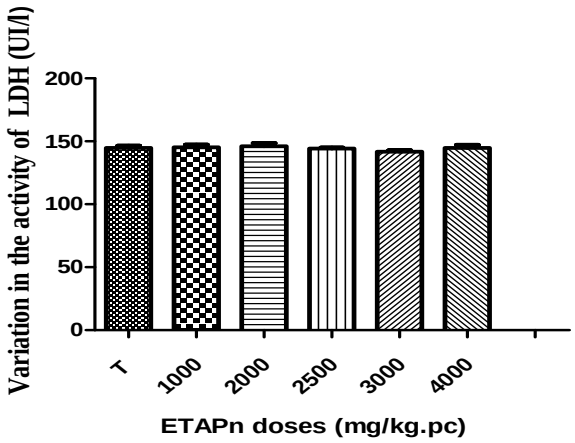

Figure 4: **Effect of different doses of *Parquetina nigrescens* on rate variation serum of Lactico dehydrogenase.**

1.5. Effect of ETAq of *Parquetina nigrescens* on the variation of the serum level of bilirubin

The results of the effects of *Parquetina nigrescens* ETAq on the variation of serum bilirubin are shown in Figure 4. The control level of bilirubin is 0.5 mg / dL. The administration to rats of 10 mg / kg bw of pheylhydrazyde increased the serum bilirubin level from 0.5 mg / dL (control value) to 1.8 mg / dL and then to 7.05 mg / dL in rats. non-anemic treated. Treatment of anemic rats with *Parquetina nigrescens* ETAq at doses of 2000 and 2500 mg / kg bw resulted in a significant decrease until serum bilirubin levels were normalized. These serum levels of bilirubin vary from 7.05 mg / dL (untreated anemic rats) to 0.6 mg / dL (rats treated with ETAq at 2500 mg / kg bw). Similar results were obtained with Bioferon 20 mg / dL, a reference antianemic drug. The results show that the level of bilirubin during treatment in anemic rats has significantly decreased.

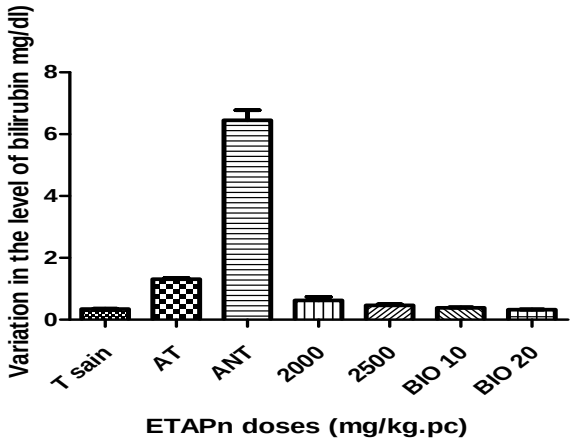

Figure 4: Effect of ETAq of *Parquetina nigrescens* on serum rate variation bilirubin.

Tsain = non-anemic control, AT = anemic control, ANT = untreated control, 2000 and 2500 = doses of aqueous extract of Parquetina nigrescens, BIO 10 and BIO 20 = Bioferon reference molecules

2. DISCUSSION

The present study is a contribution to the valorization of the African Pharmacopoeia in general and Ivorian in particular which aims to establish the scientific bases of this pharmacopoeia. The work focused on the hypoglycemic and hepatoprotective effects of the total aqueous extract (ETAq) of *Parquetina nigrescens*, a plant of the Ivorian medicinal flora traditionally used in the treatment of anemia. The results show that ETAq of *Parquetina nigrescens* contains large chemical groups such as alkaloids (very abundant), flavonoids (abundant), polyphenols, polyterpenes, tannins and sterols.

Our results indicate that increasing doses of the total aqueous extract (ETAq) of *Parquetina nigrescens* (1000 to 4000 mg / kg.pc) cause a significant drop in blood glucose. This significant decrease in blood glucose levels suggests the hypoglycemic effect of *Parquetina nigrescens* ETAq. These results confirm those of Karumi *et al* 2004. They studied the evolution of blood glucose (g / l) of the rats followed 7 hours after gavage of 1 g / kg of ethanolic extract of zygophyllum geslini coss. The blood sugar went from 1.01g / l to 0.71g / l. But this work remains preliminary and not very indicative of the actual mechanism by which the aqueous extract acts by significantly decreasing blood sugar**.**

They are grouped under the generic name "transaminases". ALT is a liver-specific enzyme in dogs, rats, rabbits, cats and primates (Farah *et al*., 2011). It can provide a quantitative assessment of the degree of damage to the liver (Al-Mamary *et al*., 2002). Our results show that *Parquetina nigrescens* ETAq did not cause liver damage as reported by Balogun et Akinloye (2012). These authors showed that the administration of the methanolic extract of Morinda morindoides did not cause any damage to the liver. The liver is a vital organ inside the body, playing a vital role in metabolic homeostasis. Evaluation of liver function can be performed by estimating the variation of these enzymes in the blood.

No significant change in the activities of LDH, a marker of cardiac function, has been observed **(**Coulibaly *et al*., 2010**)**. Lactate dehydrogenase (LDH) is an important enzyme in the metabolism of sugars, the transformation of sugars into energy, so that cells can use them. It is found in the cells of various organs and tissues: kidney, heart, muscles, pancreas, spleen, liver, brain, lungs, skin, red blood cells and placenta. In case of disease or lesion that damages cells, LDH are released into the blood stream. An increase in the level of this enzyme in the blood is indicative of severe or chronic cell damage. But further tests will be needed to discover the cause. Our results show that the *Parquetina nigrescens* ETAq, at the doses used, did not significantly alter the serum LDH values compared to the control group rats, suggesting that the *Parquetina nigrescens* ETAq did not affect the kidney, heart and liver. Our results are in agreement with those of Akinoloye *et al*. (2014) who showed that the

aqueous extract of M. morindoides does not cause any damage to the kidneys and liver.

Chemical screening (Table 1) showed that the aqueous extract of Parquetina nigrescens (Asclepiadaceae) contains alkaloids, tannins, flavonoids, polyphenols, polyterpenes and sterols. The effect of *Parquetina nigrescens* ETAq on the reduction of blood glucose levels in rats may be related to the presence of flavonoids as has been pointed out by some authors **(**N'Diaye *et al*., 2008; Olagbende-Dada *et al*., 2011**)**. Indeed, flavonoids act by improving the body's sensitivity to insulin, which reduces the incidence of type 2 diabetes **(**Lean et al 1999, Ford et al 1999).

Bilirubin is a product of the disintegration of spent red blood cells. It is absorbed and metabolized by the liver. A high level of bilirubin may mean that the liver is not functioning normally and is damaged. The results of this study show that the high level of bilirubin may be due to the degradation of red blood cells during anemia. However, the significant decrease in bilirubin during the treatment of anemia with doses of 2000 and 2500kg / kg bw, of the aqueous extract of *Parquetina nigrescens* (*Apocynaceae*), confirms the hepatoprotective activity of the plant, as **(**Talluri *et al*., 2018).

CONCLUSION

The aqueous leaf extract of *Parquetina nigrescens* (*Apocynaceae*) significantly reduced blood glucose levels in normal rats, total bilirubin in anemic rats, and without significant variation in intracellular enzymes. This action is linked to its chemical composition characterized by the presence of alkaloids, flavonoids, polyphenols, polyterpenes and sterols. The effect of this extract is reminiscent of certain secretory and hepatoprotective insulins. Subject to extensive testing, this plant species could be used in hypoglycemic and hepatoprotective treatments.

The results show that the level of bilirubin during treatment in anemic rats has significantly decreased. *Parquetina nigrescens* had no effect on Lactic-dehydrogenase (LDH), Alanine Amino Transferase (ALAT) and Aspartate Amino Transferase (ASAT), whereas blood glucose decreased significantly ($p < 0.01$).) with respect to the witness.

ETAq *Parquetina nigrescens* have hypoglycemic and hepatoprotective effects that confirm its use in traditional Ivorian medicine in the treatment of certain diseases such as diabetes.

Thanks

We thank Mr. Djih Nazaire, Assistant Professor at the Laboratory of Pharmacodynamic-Biochemistry, UFR Biosciences, University Felix HOUPHOUET-BOIGNY (Ivory Coast) for his technical assistance.

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