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Full Length Research Paper

Antisickling and toxicological profiles of leaf and stem of *Parquetina nigrescens* L.

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Folk medicine reportedly uses *Parquetina nigrescens* L. (Asclepiadaceae) as a herbal remedy for the management of sickle cell anemia. This study was carried out to screen the leaves and stem of *P. nigrescens* for antisickling activity, erythrocyte membrane -stabilizing effects and any end organ toxicity. Percentage reversal and inhibition of sickling parameters were analyzed on pre-sickled Hb^{SS} blood cell suspensions using sodium metabisulphite solution as inducer and 5 mg/ml parahydroxybenzoic acid and normal saline as positive and negative controls respectively. Effects of the plant extracts on the erythrocyte were assessed using osmotic fragility and the toxicity profile done via LD_{50} and sub-acute toxicity studies on graded concentrations of extract. Results show that *P. nigrescens* has appreciable antisickling activity, has no toxic effect when administered at low concentrations and protects the integrity of the erythrocyte membrane as evidenced in the fragiliogram by the reduction in hemolysis of the Hb^{ss} cells.

Key words: Antisickling activity, membrane integrity, osmotic fragility, *Parquetina nigrescens*, sickle cell disease.

INTRODUCTION

Sickle cell anemia is a genetic disease in which the 'SS' individual possesses an abnormal beta globin gene. A single base substitution in the gene encoding the human β-globin subunit results in the replacement of β6 glutamic acid by valine, which leads to the devastating clinical manifestations of sickle cell disease. This substitution causes a drastic reduction in the solubility of sickle cell hemoglobin (HbS) when deoxygenated (Bunn, 1997).

Under these conditions, the HbS molecules polymerize to form long crystalline intracellular mass of fibers which are responsible for the deformation of the biconcave disc shaped erythrocyte into a sickle shape A drug that prolongs the time prior to Hb^{ss} polymerization might be of therapeutic value in SCD, because a longer delay time decreases the probability of SS cell sickling. *In vitro* studies have shown that some antisickling agents, especially those of plant origin, affect the kinetics of hemoglobin (Hb) polymerization and inhibit the time course for red blood cell sickling (Iyamu et al., 2003). Recent discoveries of antisickling phytomedicines that are cheaper and less toxic alternative therapies for SCD management include *Cajanus cajan* seeds (Ekeke and Shode, 1985), Zanthoxyllum macrophylla (formerly Fagara) roots (Sofowora et al., 1975) and *Parquetina nigrescens* root extracts (Kade et al., 2003).

Though *Parquetina nigrescens* is used in herbal medicine for the management of sickle cell anemia, the anti-

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Abbreviations: Hb^{ss}, Sickle hemoglobin; **AST, ALT,** aspartate, alanine transaminase; **SCD,** sickle cell disorder; **Hb,** hemoglobin.

sickling and erythrocyte membrane stabilizing activities of leaves and stem of the plant as well as its toxicity profile have not been investigated. The study therefore sought to investigate the antisickling and erythrocyte membrane stabilizing activities, and the toxicity profile of *Parquetina nigrescens*.

MATERIALS AND METHODS

Chemicals and blood samples

Blood samples were collected with full informed consent from male and female sickle cell volunteers aged between 16 and 20 years. These individuals were in the steady state of the disease, during routine visits at the Sickle Cell out Patients' Clinic of the Lagos University Teaching Hospital, Idi-araba, Lagos, Nigeria. Sodium EDTA bottles were used for the collection and storage of the blood samples.

Diagnostic test kits for AST, ALT and Creatinine were purchased from RANDOX Company Limited, UK and the assays were performed according to the manufacturer's instructions. All other chemicals used were obtained from Sigma Chemical Company.

Plant materials

Leaves and stems of *Parquetina nigrescens* were used for the study. This plant species was identified by a plant taxonomist and confirmed at the herbarium of the Forestry Research Institute of Nigeria, Ibadan. Voucher specimen (FHI: 106998) has been deposited at the Institute's herbarium.

Extraction of the plant material

Air-dried leaves and stems of *P. nigrescens* were ground and an aliquot (400 g) was extracted by exhaustive soxhlet extraction in a method described earlier (Ogoda et al., 2002) using pet-ether (60 - 80°C) and aqueous - methanol (1:3, 60 - 80°C) as solvents. The extract was stored at 4°C in freeze - dried form and used for the antisickling experiments, osmotic fragility test and toxicological assessment.

Proximate analysis

The standard procedure as outlined in Horwitz (2000) was employed for the determination of the percentage proximate composition of carbohydrate, lipid, protein and other nutrients of the dry ground leaves and stems of *P. nigrescens* L.

Phytochemical screening

The standard procedure of Trease et al. (1996) was used to screen *P. nigrescens* extract for the presence of phenolic compounds, tannins, saponins and glycosides.

Erythrocyte membrane stability activity

The osmotic fragility of erythrocytes measures the membrane stabilizing effect of the extracts in osmotic stress/ hypotonic lysis after 30 min incubation. The protocol by (Jaja et al., 2000) was used for the analysis, with some modifications as follows. To 10 ml reaction vessels containing 4ml of different concentrations (0.00 - 0.85%) of buffered saline with pH of 7.4, 1ml of a range of concentrations of extract (1, 2, 3, 4, 5 mg/ml) and 0.05 ml Hb^{ss} blood were added. The mixture was left to incubate at room temperature for 30 min and then centrifuged at 2000 rpm for 15 min. The supernatant was collected and read at 540 nm against blank (0.85% buffered saline concentration).

Antisickling activity

In vitro screening of the herbal extracts for antisickling properties was carried out on blood samples collected from confirmed non-crisis sickle cell individuals using an earlier described method (Acquaye et al., 1982; Ekeke et al., 1985 and Ogoda et al., 2002). This involved a pre-incubation of SS blood cell suspensions with 0 - 5 mg/ml concentrations of the extracts in the presence of 2% Sodium metabisulphite solution, with para-hydroxybenzoic acid and normal saline as controls and then microscopic analysis for the time course for sickling of erythrocytes and % inhibition of hemoglobin S polymerization was done. A plot of % sickling inhibition against extract concentration was analyzed for possible explanation of the observed antisickling effect.

Toxicological profile

Experimental rats were obtained from the animal house in the college of medicine, university of Lagos, Nigeria, and research conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication # 85 - 23, 1985). Seventy Albino rats weighing 200 g were divided into seven groups with ten rats per group and various doses (2000 - 16, 000 mg/kg/d) of Carica papaya extract once, by gavage. An equivalent volume of water was administered by gavage to the rat to serve as a control. The rats were observed for 24 h and thereafter administered the same dosage of extracts for 14 days to assess sub acute toxicity. At the end of the experiment all surviving rats were euthanized by cervical dislocation under anesthesia and an aliquot of erythrocytes collected by cardiac puncture for the biochemical determination of some serum enzymes and metabolites indicative of liver and kidney functions.

These were determined using blood samples from the rats, following the manufacturer's instructions as contained in the RANDOX diagnostics test kits for transaminase (AST and ALT) and creatinine. Each rat was immediately dissected and the heart, liver, kidney and brain organs fixed in 10% phosphate buffered formal-dehyde and used for histopathological analysis; Tissue samples of the organs in 10% phosphate -buffered formaldehyde were embedded in paraffin and sections were cut and stained by a haematoxylin - eosin (HE) solution for light microscopy according to an earlier described method (Iyamu et al., 2003).

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). The students' T-test was used for comparison of the experimental groups. The level of significance was set at $p < 0.05$.

RESULTS

The aqueous - methanolic extract of *P. nigrescens* plant had a 10.87% yield and was found to contain alkaloids,

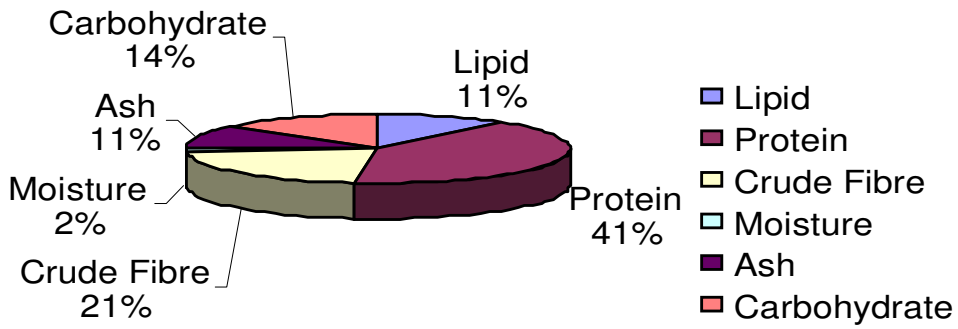

Figure 1. Percentage proximate composition of *Parquetina nigrescens* leaves and stem.

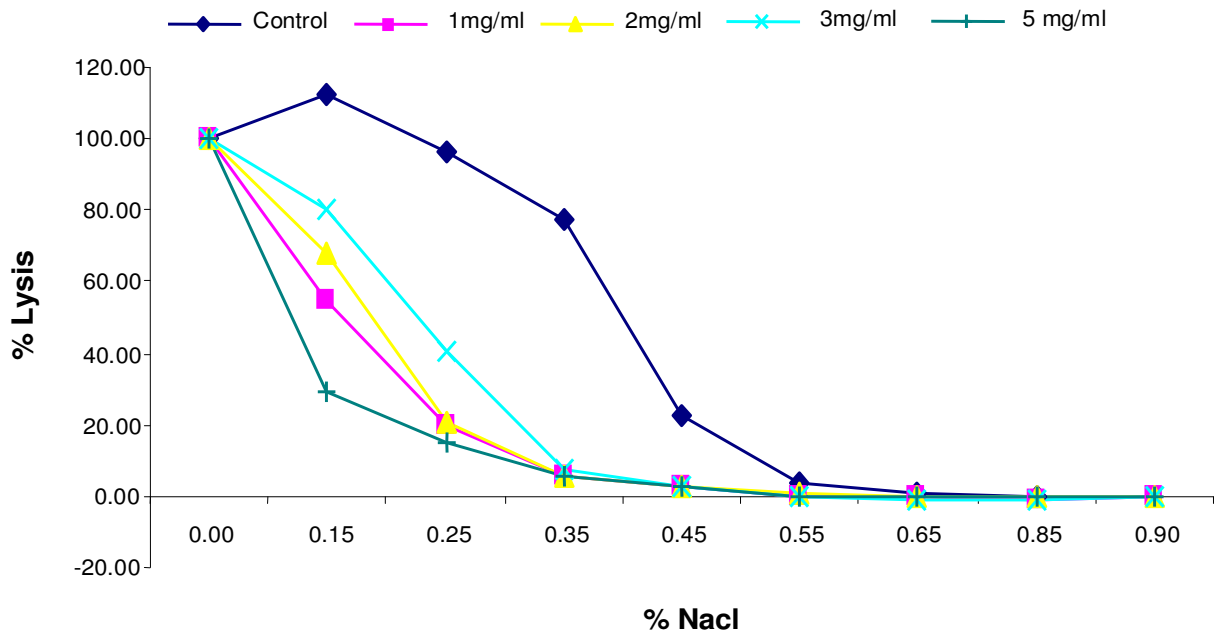

Figure 2. Osmotic fragiliogram after supplementation with various concentrations of *Parquetina nigrescens* extracts. The most effective dose observed was 5 mg/ml. The observed inhibition / reduction in RBC lysis are indicative of protective properties of the extracts on the RBC membrane, thus helping to maintain membrane integrity.

flavonoids, glycosides, cardiac glycosides, tannins, saponins and anthraquinones. Proximate analysis of the plants showed that all the macronutrients were present with protein being the most abundant (41%) (Figure 1).

Figure 2 shows the osmotic fragiliogram before and after supplementation with varied concentrations of the extract. The fragiliogram showed a marked decrease in percentage hemolysis for the 5 mg/ml extract at 0.25% buffered saline concentration.

Data from *in vitro* studies on the anti-sickling activity of the herbal extract carried out on blood samples of non-crisis sickle cell individuals, showed that pretreatment of SS cell suspensions with the extract inhibited formation of sickle cells under severe hypoxia, with only 5% sickle cells at 40 min compared with untreated SS cell suspensions which had over 65% sickle cells compared with the controls (Figure 3).

The biochemical analysis, serum transminase and creatinine levels remained steady compared with the control. Histological examination of the organs did not show any significant pathological alterations in the organs of both control and test groups.

DISCUSSION

Roots of *P. nigrescens* have been reported to have anti-sickling activity (Kade et al., 2003), but the leaves and

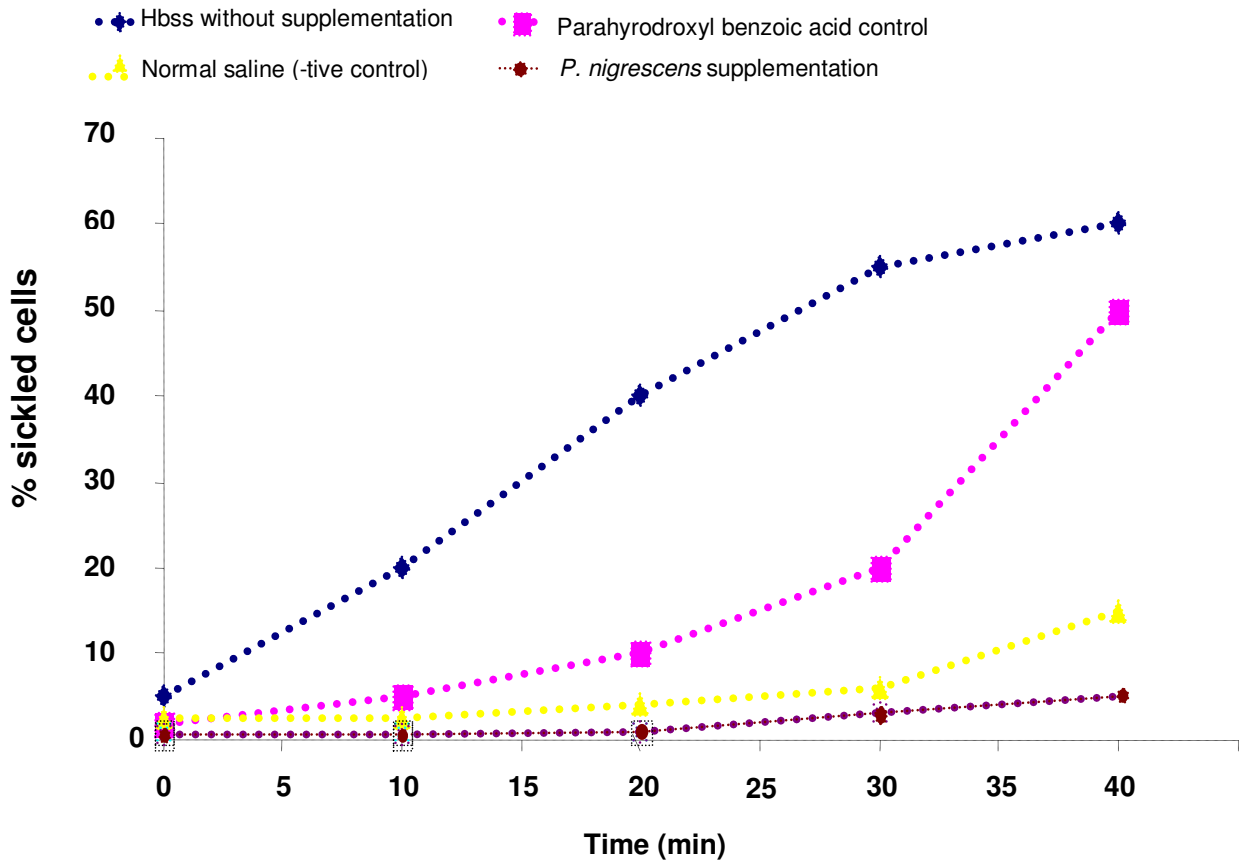

Figure 3. *In vitro* antisickling activity of 5 mg/ml concentrations of *Parquetina nigrescens* extracts. Data obtained from three independent experiments performed in duplicate using blood samples from SS patients. Sickle cell suspensions were preincubated with extracts prior to exposure to 2% sodium metabisulphite solution: as shown, Hb^{SS} supplementation with 5mg/ml Parquetina nigrescens extract reduced sickling to 5% at 40 min compared with the Hb^{SS} control which had over 65% sickled cells.

stem are also used as an indigenous herbal remedy in folk medicine for the management of anemic conditions. This necessitated the scientific evaluation of the whole plant. Phytochemical screening indicated that no anthrocyanides were present, this result as well as the histopathology findings in this study suggest that the extract was relatively non-toxic.

The effect of varied concentrations of the *Parquetina nigrescens* plant extracts on the red blood cell membrane, analyzed using the Osmotic fragility Test, revealed appreciable membrane protective effects of the herb and its inhibitory action on hemolysis of red blood cells. *P. nigrescens* leaf and stem extract was found to have an appreciable antisickling activity suggesting that the extract would be effective in prolonging the delay time of polymerization of sickle hemoglobin *in vivo* and thus affect the time course for sickling. Antisickling agents have been reported to prolong delay time of Hb polymerization as part of the mechanisms for its antisickling action (Iyamu et al., 2003). The observed antisickling activity in this study though not found to prolong the delay time in this study but definitely inhibited HbSS polymerization indicating that the extract may apply a target hit on HbS polymerization in attenuating SS cell sickling.

This observed antisickling activity of the leaves and stem of *Parquetina nigrescens* further buttresses earlier findings of Kade et al. (2003) on the antisickling activity of the roots of the plant, thus indicating that using a combination of the whole plant would be a more potent therapeutic agent in the management of sickle cell anemia.

Conclusion

Parquetina nigrescens plant extract was found to have an appreciable antisickling activity comparable to previously reported antisickling agents. The toxicity profile of the plant as assessed by histological and biochemical analyses did not reveal any end organ toxicity of the plant. These results corroborate the ethnomedical use of the plant and further indicate the feasibility of using the

leaves and stem of *P. nigrescens* as a potential candidate for SCD therapy.

REFERENCES

Acquaye CTA, Young JD, Ellory JC, Gorecki M, Wilcher M (1982). Mode of transport and possible mechanism of action of L-Phenylalamine benzylester as an antisickling agent. Biochimica Biophysica Acta. 693: 407-416.

Bunn FH (1997). Pathogenesis and Treatment of Sickle Cell Disease. The New Eng. J. Med. 337: 762-769.

Ekeke GI, Shode FO (1985). The Reversion of Sickled cells by *Cajanus cajan*. Planta med. 6: 504-507.

Horwitz W (2000). Official Methods of Analysis of AOAC International, 17th Edition. USA. pp. 30-59.

Iyamu EW, Turner EA, Asakura T (2003). Niprisan (Nix – 0699) improves the survival rates of transgenic sickle cell mice under acute severe hypoxic conditions. Brit. J. Hematol. 122: 1001-1008.

Jaja SI, Kehinde MO, Gbenebitse S, Mojiminyi FBO, Ogungbemi AI (2000). Effect of vitamin C on arterial blood pressure, irreversible sickled cells and osmotic fragility in sickle cell anemia subjects. Niger. J. Physiol. Sci. 16(1-2): 14-18.

Kade IJ, Kotila OO, Ayeleso AO, Olaleye AA, Olawoye TL (2003). Antisickling properties of *Parquetina nigrescens*. Biomed. Res. (Alligarh). 14: 185-188.

Ogoda OJ, Akubue PI, Okide GB (2002). The Kinetics of Reversal of Pre-sickled Erythrocytes by the Aqueous Extract of Cajanus Cajan seeds. Phytother. Res. 16: 1-3.

Sofowora EA, Issacs-Sodeye NA, Ogunkoya LO (1975). Antisickling properties of Fagara. Lloydia 38: 169-171.