Research Article (Open access)

Invitro Anti-Inflammatory Activity of *Parkia biglobosa* Fruit Bark Extract

***Ukwuani AN[1], Ahmad HB[1]**

[1]Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, Aleiro, Kebbi State P.M.B, Nigeria

Email:pinknnenna@yahoo.com

ABSTRACT- The invitro anti-inflammatory activity of various solvent fractions of *Parkia biglobosa* fruit bark was investigated using human red blood cell membrane stabilization, heat-induced hemolysis and protein denaturation methods. All the extracts of *P. biglobosa* fruit bark showed a concentration dependent increase in anti-inflammatory activity. The anti-inflammatory activity of the crude extract (60.8%, 58.3%, 78.2%) and last remaining aqueous extract (61.1%, 54.1%, 77.2%) have the maximum membrane stabilization, protection against hemolysis and albumin denaturation respectively which was comparable to Diclofenac sodium (61.4%, 60.6%, 100%) at 400μg/ml concentration. This study suggests that *P. biglobosa* fruit bark posses enough potential to reduce inflammation, hence directs the importance of further research and development of novel anti-inflammatory agents.

Key words- Invitro anti-inflammatory, fruit bark, HRBC membrane stabilization, hemolysis, protection

---IJLSSR---

INTRODUCTION

Inflammation is the reaction of living tissue to injury, infection or irritation. It involves complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair [1] which is aimed at host defence and usually activated in most disease condition. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. Due to risk of adverse effects encountered with the use of synthetic anti-inflammatory drugs, natural products derived from plants may offer an alternative source of anti-inflammatory agent. The use of traditional medicine and medicinal plants in most developing countries, as a normo-tive basis for the maintenance of good health, has been widely observed [2].

Parkia biglobosa is widely acclaimed by the Hausa communities of northern Nigeria for the treatment of diseases such as malaria, diabetes mellitus and pains. The stem barks is boiled in water and taken as a decoction for the treatment of malaria, inflammatory diseases and infections to diarrhea [3]. The bark soaked in ethanol is also used in some co--mmunities for anti-diarrhoeal properties and as effective anti- snake venoms that protects against neurotoxic, haemotoxic and cytotoxic effects of poisonous snakes [4]. Also, the leaves, fruits and seeds of *P. biglobosa* have also been used to manage various diseases [5].

**Address for Correspondence:*
**Ukwuani AN*
Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, Aleiro, Kebbi State. P.M.B, 1144
Email:pinknnenna@yahoo.com

Received: 31 July 2015/Revised: 16 August 2015/Accepted: 30 August 2015

MATERIALS AND METHODS

Plant material collection and preparation

The fruit back of *P. biglobosa* were collected in April, 2014 from Bimin Yauri Town, Ngaski Local Government Area, Kebbi State, Nigeria. The plant was identified by their local names and later authenticated by the herbarium at Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero with a voucher specimen (No 281). Fresh fruit bark were air-dried and grinded into coarse powder using mortar and pestle.

Extraction of the plant material

Crude extraction was carried out according to the method of Edeoga *et al* [6] with slight modifications. One kilogram (1.0 kg) of the fruit bark powder was extracted with hydromethanolic solvent (methanol (70): water (30)) for 72 hours. This was then filtered and evaporated to dryness in an oven at 45°C to give hydromethanolic crude extract (346g).

Phytochemical analysis

Preliminary phytochemical studies were conducted as per standard procedures described by Trease and Evan [7] and Harbone [8]

Solvent Partitioning of the Methanolic Extract

Hydromethanolic extract (100g) was redissolved in distilled water (200 mL) in a separating funnel and partitioned with *n*-hexane (4 × 200 mL). The resulting *n*-hexane phase was concentrated to dryness and known as hexane fraction which was stored in a freezer in an air-tight container.

Copyright © 2015 | IJLSSR by Society for Scientific Research is under a CC BY-NC 4.0 International License

The resulting aqueous phase was further partitioned with chloroform, ethyl acetate and butanol respectively. The last remaining aqueous fraction was then evaporated to dryness and kept for further use in the freezer in an air-tight container [6].

Invitro anti inflammatory studies

Preparation of Red Blood cells (RBCs) suspension

Fresh whole human blood (5mL) was collected and transferred to the centrifuged tubes containing Heparin or EDTA or Sodium citrate to prevent clotting. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline [9].

Human red blood cell (HRBC) membrane stabilization method

The reaction mixture consist of 1.0ml of test sample of different concentration (50-400μg/ml) in normal saline and 0.5 ml of 10% HRBC suspension, 1 ml of 0.2 M phosphate buffer, 1 ml hypo saline were incubated at 37C for 30 min and centrifuged at 3000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac sodium was used as standard and a control was prepared without extracts [10]. The percentage of hemolysis and membrane stabilization or protection was calculated by using the following formula;

% of Hemolysis = (Optical density of test sample / Optical density of control) X 100

% Protection = 100 – [(Optical density of test sample / Optical density of control) X 100]

Heat induced human red blood cell (HRBC) membrane stabilization method

The reaction mixture in heat induced hemolysis consists of 1.0 ml of test sample of different concentrations (50μg – 400μg/ml) in normal saline and 1.0 ml of 10% RBC suspension. Diclofenac sodium was taken as a standard drug. Control was prepared by distilled water instead of normal saline to produce 100 % hemolysis without plant extracts. All the tubes containing reaction mixture were incubated in a water bath at 56^0C for 30 min. After incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm [11]. The experiment was performed in triplicates. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following Formula:

% of Hemolysis = (Optical density of test sample / Optical density of control) X 100

% Protection = 100 – [(Optical density of test sample / Optical density of control) X 100]

Albumin denaturation method

Protein denaturation was performed as described by Elias *et al*. [12] with slight modifications. The reaction mixture consists of 1.0 ml of distilled water containing varied concentrations of plant extracts or standard (50μg – 400 μg), 0.2 ml of .05 % BSA and 1.8 ml of 0.2 M phosphate buffered saline (pH 6.4). The mixtures were incubated at 37ºC for 15 minutes and then heated at 70ºC for 5 minutes. After cooling, the absorbance was measured spectrophotometrically at 660nm against a blank. Diclofenac sodium was used as standard drug and the percentage inhibition of protein denaturation was calculated by using the following formula;

% Inhibition=100 ×[Vt/Vc-1]

Where, Vt = absorbance of test sample; Vc = absorbance of control.

RESULTS AND DISCUSSION

Percentage Yield and Phytochemistry

The percentage yield of 1000g of *P. biglobosa* fruit bark using hydro-methanolic extraction was 34.6% while fractionation of 100g of hydro-methanolic extract of *P. biglobosa* fruit bark obtained percentage yield as presented in Table 1. Phytochemical screening which revealed the present of secondary metabolites as shown below in Table 2.

Table 1: Percentage yield of *P. biglobosa* fruit bark (100g)

Fraction	*Percentage yield (%)*
Hexane	28.6
Chloroform	23.5
Ethyl acetate	21.2
Butanol	4.4
Last remaining aqueous fraction	14.8

Table 2: Preliminary Phytochemical screening of *P. biglobosa* fruit bark

Phytochemicals	*Result*
Alkaloids	+
Tannins	++
Saponins	ND
Flavonoids	+++
Steroids	++
Cardiac glycosides	+
Reducing sugar	++

(+) trace amount, (++) moderately present, (+++) highly present, (ND) Not Detected

Copyright © 2015 | IJLSSR by Society for Scientific Research is under a CC BY-NC 4.0 International License

Membrane stabilization activity of *P. biglobosa* fruit bark

Lysosomal enzymes released during inflammation produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [13]. The invitro anti-inflammatory activity of *P. biglobosa* fruit bark fractions were assessed by HRBC membrane stabilization method (Fig. 1). Crude extract and fractions of *P. biglobosa* fruit bark exhibited varying degree of anti-inflammatory activity which was comparable to the standard drug Diclofenac sodium. The result showed a concentration dependent increase in percentage protection of the membrane in all the tested fractions.

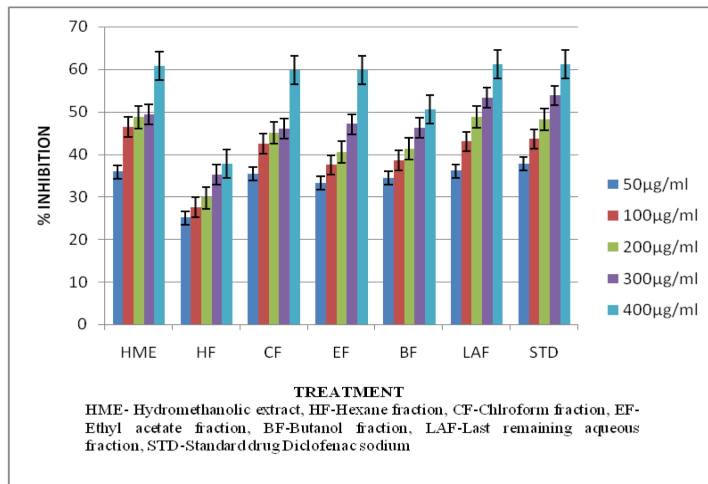

Fig. 1: Membrane Stabilization Effect of *Parkia biglobosa* fruit bark

Since the human red blood cell membrane is analogous to the lysosomal membrane [14], its stabilization indicates the stabilization of lysosomal membranes. Stabilization of the membranes of these cells inhibits lysis and subsequent release of the cytoplasmic contents which in turn limits the tissue damage and exacerbation of the inflammatory response [15]. In this study, *P. biglobosa* fruit bark was able to confer a significant protection of HRBC membrane when compared to Diclofenac sodium.

Inhibition of Heat-induced hemolysis

Exposure of red blood cells to hypotonic medium, heat, injurious substances such as methyl salicylate or phenylhydrazine results in the lysis of membranes accompanied by haemolysis and oxidation of haemoglobin [16]. The effect of *P. biglobosa* fruit bark on hypotonicity-induced hemolysis is presented in Fig. 2. At the highest dose (400µg/ml), hydromethanolic crude extract and last remaining aqueous fraction was capable of protecting the membrane by 60.8% and 61.1% which was not significantly different from the standard drug Diclofenac sodium. The prevention of heat-induced HRBC membrane lysis is taken as measure of anti-inflammatory activity of *P. biglobosa* fruit bark.

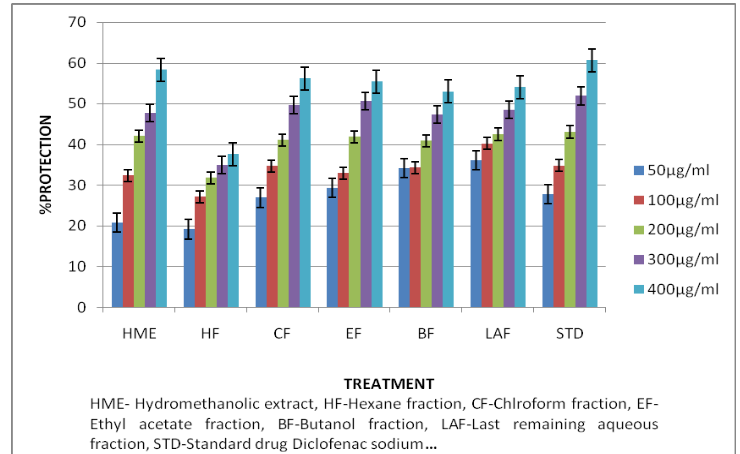

Fig. 2: Effect of *Parkia biglobosa* Fruit Bark on Heat-Induced Hemolysis

Albumin Denaturation

Protein denaturation is the process in which protein lose their secondary and tertiary structure by application of external stress or compounds such as strong acid or base, organic solvent or heat [17]. Denaturation of protein is a well documented cause of inflammation. In the present study, *P. biglobosa* fruit bark was effective in inhibiting albumin denaturation (Fig. 3). Maximum inhibition of 78.2% and 77.2% was observed in hydromethanolic extract and last remain aqueous fraction respectively at a concentration of 400µg/ml. On the other hand, Diclofenac sodium, the standard anti-inflammatory drug showed a maximum inhibition of 100%. The increased percentage of inhibition of albumin denaturation expresses the anti-inflammatory efficacy of *P. biglobosa* fruit bark.

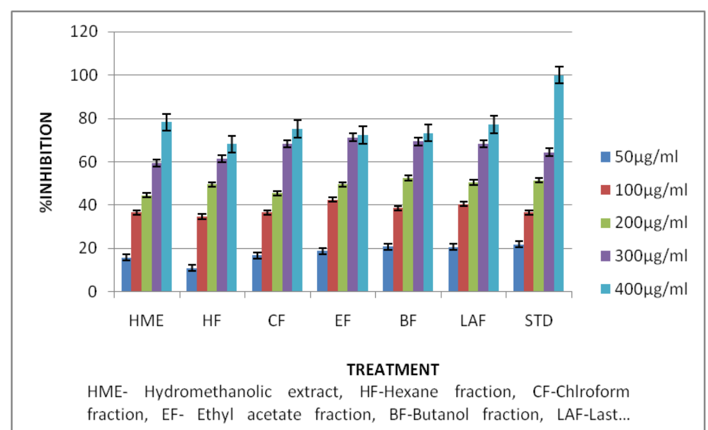

Fig. 3: Effect of *Parkia biglobosa* Fruit Bark on Albumin Denaturation

CONCLUSIONS

The present study indicates that the different solvent extract of *P. biglobosa* fruit bark possess anti-inflammatory property. This activity may be due to presence of phenolic compounds such as flavonoids and tannins. This invitro study is a preliminary evaluation of anti-inflammatory activity of *Parkia biglobosa* fruit bark and validates

Copyright © 2015 | IJLSSR by Society for Scientific Research is under a CC BY-NC 4.0 International License

the folkloric use of this plant in the treatment of inflammatory conditions. Further research work to evaluate in vivo activity on animals, isolation and characterization of its bioative components is recommended.

REFERENCES

[1] Vane JR, Bottling RM. New insights into the model of action of anti-inflammatory drugs. *Inflamm. Res. 1991*; 44(1): 1 – 10.

[2] UNESCO (1996). *Culture and Health*, Orientation Texts– World Decade for Cultural Development 1988–1997, Document CLT/DEC/PRO – 1996, Paris, France, pp. 129.

[3] Asase A, Alfred A, Yeboah O, Odamtten GT, Simmonds SJ. Ethnobotanical study of some Ghanaian antimalarial plant. *J. Ethnopharmacol*. 2005; 99: 273-279.

[4] Agunu A, Yusuf S, Andrew O, Gabriel U, Abdulkadir M. *et al*. Evaluation of five medicinal plants used in diarrhea treatment in Nigeria. *J. Ethnopharmacol* 2001; 101: 27-30.

[5] Builders MI, Aguiyi JC. Toxicity studies of the extracts of *Parkia biglobosa* stem bark in rats. *Brit. J. Pharmaceut. Res.* 2012; 2(1): 1-16.

[6] Edeoga HO, Okwu DE, Mbaebe BO. Phytochemical constituent of some Nigerian medicinal plants. *Afr. J. Biotechnol.* 2005, 685–688.

[7] Trease GE, Evans WC. Pharmacognosy. 13th (ed). ELBS/Bailliere Tindall, London. 1989; 345-6, 535-6, 772-3.

[8] Harborne JB. Phytochemical Methods. Chapman and Hall Ltd., London, UK, 1973; pp: 49-188.

[9] Sadique J, Al Raqobah WA, Bugharith ME and El-Gindy AR. (1989).The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. *Fitoterapia*. 1989; 60 (6): 525-532.

[10] Gandhisan R, Thamaraichelvan A, Baburaj A. Anti-inflammatory action of *Lanneacoromandelica* HRBC membrane stabilization. *Fitotherapia.* 1991; 62:82- 83.

[11] Shinde U A, Phadke AS, Nair AM, Mugantiwar AA, Dikshit VJ and Saraf VO. Membrane stabilizing activity - A possible mechanism of action for the anti-inflammatory activity of *Cedrusdeodara* wood oil. *Fitoterapia. 1999;* 70 (3): 251-257.

[12] Elais G, Rao MN. Inhibition of albumin denaturation and anti-inflammatory activity of dehydrozingerone and its analogs. *Indian J. Exp. Biol.*1988; 26(10): 540 – 543.

[13] Rajendran V, Lakshmi KS. In vitro and in vivo anti inflammatory activity of leaves of *Symplocosco chinchinensis* (Lour) Moore. *Bangladesh J Pharmacol*.2008; 3; 121-124.

[14] Chou CT. The anti-inflammatory effect of *Tripterygium wilfordii* Hook on adjuvant induced paw edema in rats and inflammatory mediators release. *Phytother Res*. 1997; (11):152-154.

[15] Charles OO, Peter AA, Nkemjika JO, Theophine CN, Anthonia CO, Chukwuemeka SN. "*Acanthus montanus*: An experimental evaluation of the antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles". *BMC Complementary and Alternative Medicine.* 2008; (8): 27.

[16] Ferrali M, Signorni C, Ciccoli L, Comporti M. (1992).Iron release and membrane damage in erythrocytes exposed to oxidizing agents phenylhydrazine, divicine and isouramil. *Biochemical Journal.* 1992*;* 285 (1) 295-301.

[17] Megha GC, Bhoomi BJ, Kinnari NM. In Vitro Antidiabetic and Anti-inflammatory activity of Stem Bark of *Bauhinia Purpurea*. *Bull. Pharmaceut. Med. Sci.* 2013; 1(2): 139 – 150.

Copyright © 2015 | IJLSSR by Society for Scientific Research is under a CC BY-NC 4.0 International License