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Screening of ethyl acetate extract of *Bridelia micrantha* for hepatoprotective and anti-oxidant activities on Wistar rats

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ABSTRACT

Objective: To explore the hepatoprotective and anti-oxidant activities of the methanolic leaf extract of *Bridelia micrantha* (*B. micrantha*) on paracetamol induced liver damage in Wistar rats. **Methods:** Parameters were measured including alanine aminotransaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin and total protein. The anti-oxidant effects were studied using the 1, 1-Diphenynl-2-Picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assay methods. **Results:** *B. micrantha* extract decreased the level of AST in the rats given PCM from (129.47±0.92I) IU/L to (57.78±1.71) IU/L (P<0.05). This was lower than the value for Silymarin which was (59.92±1.41) IU/L. ALT concentration was reduced from (150.18±2.23) IU/L to (79.10±2.01) IU/L (P<0.05). ALP was reduced from (49.86±0.85) IU/L to (29.64 ±1.53) IU/L (P<0.05). Total bilirubin was reduced from (2.14±0.10 mg/dL) to (0.18±0.07) mg/dL (P<0.05) while total protein was increased from (4.26±0.30) mg/dL to (6.20±0.19) mg/dL (P<0.05). Concentrations ranging from 10 - 400 μg/mL of *B. micrantha* were assayed for antioxidant activities. The DPPH assay showed 98% antioxidant activity at concentration of 400 μg/mL. The FRAP values were 0.016, 0.39, 0.455, 0.601 and 1.382 μM at 10, 50, 100, 200 and 400 μg/mL respectively. **Conclusions:** Results suggest that *B. micrantha* has hepatoprotective and anti oxidant potentials. However, further work involving fractionation needs to done to isolate the active compound responsible for the hepatoprotective activity.

1. Introduction

It is believed that approximately 80% of the third world population is almost entirely dependent on traditional medicine[1]. Application of phytotherapeutic medication especially in traditional medicine is a recognized profession and has played an important role as an alternative Medicare which involves direct use of plants for preventing and healing diseases and infections[2].

Bridelia micrantha (*B. micrantha*) belongs to the Family Euphorbiaceae and is known as coast gold in English. The Igbo people of Southern Nigeria call it Ogaofia, Asha; the Yorubas call it Idaodan. Swahilis call it Mkarakaka. It is known as Musabayembe by the Bemba tribe in central Africa. Yoruba herbalists of western Nigeria use the bark for bringing full-term prolonged pregnancy[3]. In Ghana, a leaf decoction is given to expel guinea worm[4], and in Ivory Coast, it is a powerful purgative in cases of obstinate constipation and poisoning[5]. It is administered in various preparations in Senegal for stomach and intestinal complaints, sterility, oedema and in combination with other drug plant for shock.

Paracetamol is a mild analgesic and antipyretic agent which is safe and effective when taken in low doses Ingestion of high doses leads to acute liver failure accompanied by centri-lobular degeneration and necrosis in the liver of both man and experimental animals[6]. Toxicity of paracetamol is thought to be produced by N-acetyl-p-benzoquinine imine, a reactive electrophilic metabolite of a cytochrome P-450 mediated reaction.

The present study is therefore designed to investigate the hepatoprotective and anti oxidant effects of the ethyl acetate leaf extract of *B. micrantha*.

2. Materials and methods

2.1. Preparation of plant material

The fresh leaves of *B. micrantha* were obtained and identified by Mr. Ozioko, a taxonomist with Bioresources

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Development and Conservation Programme (BDCP). They were dried at room temperature and pulverized into powder with a laboratory mill. The powder (1 000 g) was exhaustively extracted with ethyl acetate. The extraction was treated by cold maceration at 37 ℃ with intermittent shaking for 48 h. The extract was concentrated by vacuum rotary evaporator and stored in a refrigerator at 4 ℃.

2.2. Experimental animals

Wistar rats of both sex with weighing of 86-128 g were used as test animals. The animals were housed in cages at room temperature under light period of 16-18 h daily. All animals were fed *ad libitum* with Vital® pelleted feed and water.

2.3. Solutions, reagents, chemicals and equipment

Freshly prepared solutions and analytical grade chemicals were used in all the experiments. Alloxan monohydrate (Sigma-Aldrich, Germany), 2, 2-diphenyl -1- picryl hydrazyl (Sigma-Aldrich, Germany), 2, 4, 6- tri (2-pyridyl)-s- triazine (Fluka), ascorbic acid (Hopkins and Williams Ltd), Paracetamol (Emzor), Silymarin, (Roche, Germany), spectrophotometer (Spectrumlab, USA).

2.4. Acute toxicity study

Six groups of albino rats of both sexes with each group containing five rats were used, bringing the total to 30 rats. Five of the groups were treated orally with varying doses of the *B. micrantha* extract at 500, 1 000, 1 500, 2 000 and 3 000 mg/kg, respectively. The sixth group was given an equivalent volume of distilled water to serve as control. The animals were observed for toxic signs like excitability, dullness, diarrhea, inappetence and death over 72 hours.

2.5. Paracetamol-induced hepatotoxicity

Thirty six Wistar rats were used for the experiment. The animals were divided into six groups of six rats per group. Group one received distilled water for 14 days and served as normal control (no challenge with paracetamol). All other groups were challenged with a single dose of 2 000 mg/kg of paracetamol, *per os* prior to treatment which was done 12 h after paracetamol administration. Group two received paracetamol only (negative control). Group three received silymarin (25 mg/kg) for 14 days. Groups four, five, and six received 75, 150 and 300 mg/kg of the ethyl acetate leaf extract of *B. micrantha* respectively for 14 days. After 12 h, blood samples for biochemical studies were collected through the lacrimal vein of the rats into marked sample bottles. These were allowed to stand for 45 mins at room temperature. The serum was separated using a Pasteur pipette into sterile serum sample tubes from where they were drawn for biochemical assays. The method of Reithman and Frankel[7] was adopted for the alanine aminotransaminase (ALT) and aspartate aminotransferase (AST) assays. Alkaline phosphatase actvity (ALP) was estimated using the method of King EJ and King PR[8]. Bilirubin was determined using the method Tietz[9]. Total protein was estimated using the method of Johnson[10].

2.6. Estimation of the antioxidant activity of B. micrantha using the 1, 1-Diphenynl-2-Picrylhydrazyl (DPPH) antioxidant assay method

The method of Mensor[11] was adopted. 2 mL of test extract at concentrations of 10, 50, 100, 200 and 400 μg/mL was each mixed with 1 mL of 0.5 mM DPPH (in methanol). Absorbance at 517 nm was taken after 30 minutes incubation in the dark at room temperature. The concentrations were prepared in triplicates. The percentage antioxidant activity was calculated as follows:

% antioxidant activity[AA] = 100 −([absorbance of sample− absorbance of blank] × 100)

Absorbance of control

1 mL of methanol plus 2 mL of the extract was used as blank while 1 mL of 0.5 mM DPPH solution plus 2 mL of methanol was used as control. Ascorbic acid was used as reference standard.

2.7. Estimation of the antioxidant activity of B. micrantha using the Ferric Reducing Antioxidant Power FRAP assay method.

The total antioxidant potential of sample was determined using a ferric reducing ability of plasma FRAP assay of Benzie and Strain as a measure of "antioxidant power"[12]. All determinations were performed in triplicates.

2.8. Data analysis

Data are expressed as mean±SEM and were analyzed for significance by Dunnet's test using the SPSS 16 software. In all cases, the criterion for statistical significance was $P<0.05$.

3. Results

The ethyl acetate leaf extract of *B. micrantha* was brownish green in colour. The total solid recovered from the extraction was 115.28 g. No death was recorded in the rats treated orally with varying doses (500 − 3 000 mg/kg) of the extract within 72 h. However, rats treated with 3 000 mg/kg of the extract showed transient dullness, which disappeared 1 h after the administration of the extract. Results of the hepatoprotective study (Table 1) showed that *B. micrantha* had a positive dose dependent influence on the level of AST in rats, with the dose of 300 mg/kg being better than the results with Silymarin. It caused a decrease in the level of AST in the rats given PCM from (129.47±0.92) IU/L to (57.78±1.71) IU/L ($P<0.05$). This was lower than the value for Silymarin which was (59.92±1.41) IU/L. ALT concentration was reduced from (150.18±2.23) IU/L to (79.10±2.01) IU/L ($P<0.05$). ALP was reduced from (49.86±0.85) IU/L to (29.64±1.53) IU/L ($P<0.05$).

Total bilirubin was reduced from (2.14±0.10) mg/dL to (0.18±0.07) mg/dL ($P<0.05$). Total protein was increased from (4.26±0.30) mg/dL to (6.20±0.19) mg/dL ($P<0.05$). The extract of *B. micrantha* had an increasing percentage antioxidant activity with increasing concentrations after 30 min incubation in the dark. This was compared with DPPH assay of L-ascorbic acid (Figure 1). At the concentration of 400 μg/mL, *B. micrantha* had a 97.7% antioxidant activity while ascorbic acid had 79.98%. The FRAP values were 0.016, 0.390, 0.455, 0.601 and 1.382 μM at 10, 50, 100, 200 and 400 μg/mL respectively. The extract of B. micrantha showed increased ferric reducing antioxidant power which was dependent on the concentration of the extract showing the highest FRAP value at 400 μg/mL (Figure 2).

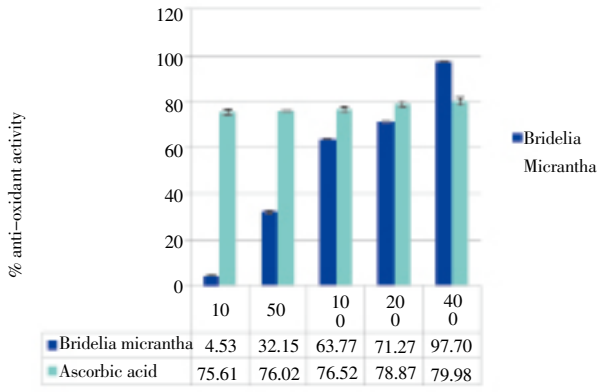

Figure 1. DPPH antioxidant activity of *B. micrantha*.

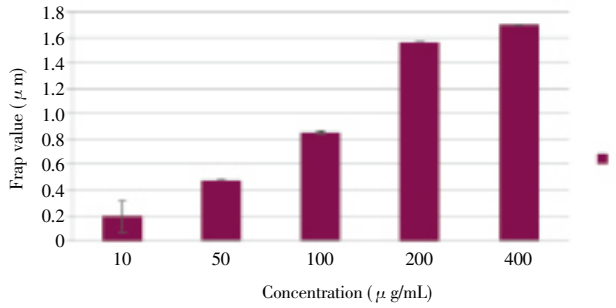

Figure 2. FRAP assay of *B. micrantha*.

Table 1

The effect of the ethyl acetate leaf extract of *B. micrantha* on biochemical parameters.

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dL)	Total protein (mg/dL)
Normal control	55.15±3.31[a]	78.13±3.10[a]	28.38±2.73[a]	0.17±0.01[a]	6.53±0.18 [a]
PCM only	129.47±0.92	150.18±2.23	49.86±0.85	2.14±0.10	4.26±0.30
Silymarin+PCM	59.92±1.41[a]	82.54±1.52[a]	31.03±1.50[a]	0.18±0.03 [a]	6.19±0.16 [a]
Extract (75 mg/kg) +PCM	124.83±1.20	133.35±1.65[a]	37.81±1.11[a]	2.00±0.30	5.21±0.25 [a]
Extract(150 mg/kg) + PCM	82.72±1.99[a]	119.68±1.10[a]	30.09±1.60[a]	0.20±0.19 [a]	5.61±0.20
Extract (300 mg/kg)+ PCM	57.78±1.71[a]	79.10±2.01[a]	29.64±1.53[a]	0.18±0.07 [a]	6.20±0.19 [a]

[a] means significantly different from group treated with only PCM $P<0.05$.

4. Discussion

The absence of signs of toxicity and death in rats treated with the extract showed that it is relatively safe and explains why this plant has a wide acceptance in traditional medicine practice. Ethyl acetate extract of *B. micrantha* had hepatoprotective effects on the Wistar rats especially at 300 mg/kg, which were better than that of the known Silymarin. Thus, this plant has the ability to help in the regeneration of damaged hepatic cells and will be very useful in the treatment of hepatic diseases, being safe, cheap and readily available[7]. The results of the antioxidant assays (DPPH and FRAP) showed that the methanol leaf extract of *B. micrantha* has antioxidant properties. It was able to scavenge the free radicals produced by DPPH, and also reduce ferric oxide appreciably. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe11-tripyridyltriazine compound from colorless oxidized Fe111 form by the action of electron donating antioxidants.

It can be concluded from the results that the oral administration of the ethyl acetate leaf extract of *B. micrantha* is beneficial in improving the antioxidant status of the body since its DPPH antioxidant assays showed better free radical scavenging activity than L-ascorbic acid[9]. The experiment also revealed that ethyl acetate extracts of *B. micrantha* has a better hepatoprotective capacity than Silymarin. Further pharmacological and biochemical investigations are going on to find out the mechanism of action and also identify the active compounds responsible for the hepatoprotective and antioxidant activities respectively.

Conflict of interest statement

We declare that we have no conflict of interest.

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