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Bryophyllum pinnatum inhibits arginase II activity and prevents oxidative damage occasioned by carbon tetrachloride (CCl_4) in rats

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ABSTRACT

Bryophyllum pinnatum (*B. pinnatum*) (Lam.) Oken is used in tropical Africa for the treatment of several diseases such as kidney and urinary disorders. This study was aimed to evaluate the effect of *B. pinnatum* on arginase II activity and its prevention against renal oxidative damage occasioned by CCl_4 in rats. Rats were randomly divided into six groups; group I served as the control, group II served as carbon tetrachloride (CCl_4) intoxicated group, group III–V animals were pre-treated with silymarin (25 mg/kg body weight), 25 mg/kg body weight aqueous extracts of *Bryophyllum pinnatum* (AEBP) and 50 mg/kg body weight AEBP, respectively, for 14 days, followed by a single injection of CCl_4. Group VI rats received AEBP only (50 mg/kg body weight). Results obtained revealed that CCl_4 intoxication significantly increased ($p < 0.05$) the levels of renal markers (serum urea, creatinine and arginase II) in rats when compared to the control group. Further, oxidative stress status appeared in CCl_4-intoxicated rats, as evidence by significant elevation in malondialdehyde (MDA), with concomitant decrease in levels of functional sulfhydryl groups (−SH), antioxidant enzymes and nitric oxide in rats' kidney. These adverse changes, due to CCl_4 intoxication in rats, were however, prevented by pre-treatment with AEBP leaves (25 and 50 mg/kg body weight). The inhibition of arginase II, as well as increased antioxidant status by AEBP in CCl_4-intoxicated rats suggests that *B. pinnatum* can protect kidney against CCl_4-induced oxidative damage.

1. Introduction

The kidney is an important organ performing a dynamic role in the regulation and control of homeostasis subject to absorptive, secretory, endocrine and metabolic functions, as well as the removal of excess organic molecules and toxins from the blood [1]. These functions are affected in kidney pathology, which may be associated with oxidative stress. The oxidation of polyunsaturated fatty acids, a major component of membrane lipids, is responsible for tissue damage, a process caused by reactive oxygen species (ROS) [2]. Overproduction of ROS, when greater than the body's antioxidative capability results in oxidative stress, and this imbalance leads to damage of cellular components such as proteins, lipids, and nucleic acids [3]. This also affects some physiological adaptations such as loss of energy metabolism, cell cycle control, and regulation of intracellular signal transduction [4].Oxidative stress is associated with numerous diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, chronic kidney disorder, alcoholic liver disease, aging, and cancer [5]. Toxic chemicals or xenobiotics, such as CCl_4, are now known to exert toxic effects *via* free radical–mediated mechanism.

Carbon tetrachloride is a widely used and powerful environmental toxicant. Meftah et al. [6] reported that CCl_4 initiate lipid peroxidation, which consequently damage body organs, such as the kidneys, liver, heart, testis and brain. These changes occur as a result of bio-activation of CCl_4 into trichloromethyl radical ($\cdot CCl_3$) and trichloromethyl peroxy radical ($\cdot OOCCl_3$) by cytochrome P450 system, which target vital molecules such as DNA, lipids, protein and carbohydrates.

Medicinal plants and products serve as the basis of health care throughout the world since the earliest days of humanity and are still widely used [7]. Globally, 80% of the world population is estimated to exclusively rely on plants and plant products as source of medicine [8,9]. Medicinal plants products are prescribed extensively because of their effectiveness, low cost, availability and lesser side effect [10]. Further, investigations into the chemical and biological activities of

Abbreviations: AEBP, aqueous extracts of *Bryophyllum pinnatum*; *B. pinnatum*, *Bryophyllum pinnatum*; CAT, catalase; CCl_4, carbon tetrachloride; CYP2E, cytochrome p450 isoform; eNOs, endothelium nitric oxide synthase; GPx, glutathione peroxidase; GSH, glutathione reduced; GST, glutathione *S*- transferase; MDA, malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase; TSH, total protein thiol; NPSH, non-protein thiol

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plants have led to the emergence of medicinal chemistry as a major route for the development of novel and more effective therapeutic agents [11].

B. pinnatum popularly called "Resurrection plant, life plant or miracle plant" belongs to the crassulaceae family [12]. It is a perennial herb growing widely in tropical, sub-tropical and warmer temperate regions. It is used in folklore medicine in tropical Africa, tropical America, India, China and Australia [13], and flourishes throughout the Southern part of Nigeria [14]. *B. pinnatum* possesses secondary metabolites with a wide range of active compounds, including alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, phenols, anthocyanins, quinines, carotenoids, tocopherol, lectins, lipids and organic acids [15–17]. Previous research revealed that the plant has been used in traditional medicine for the treatment of diseases, such as hypertension, kidney and urinary disorder, and cough [18]. The leaves possess a variety of medicinal properties, including antimicrobial, antiulcer, antihypertensive, tocolytic [19], wound healing, antidiabetic, anti-inflammatory, analgesic [17,20,21] antidepressant [22], antitumor [23]. There is however, limited information on the use of *B. pinnatum* leaves against renal damage in rats. This study was therefore designed to evaluate the effect of *B. pinnatum* on arginase II activity and its prevention against renal oxidative damage occasioned by CCl_4 in rats.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals, such as carbon tetrachloride, perchloric acid, thiobarbituric acid, trichloroacetic acid, hydrogen peroxide, dithionitrobenzene, and adrenaline were purchased from Sigma chemical co. (St. Louis, MO, USA). Ethanol, acetic acid, sodium carbonate, sodium azide, potassium dichromate, Tris–HCl buffer, sodium dodecyl sulphate, and ascorbic acid were sourced from BDH chemicals Ltd., (Poole, England). Pharmaceutical grade silymarin under the brand name "Silybon" was purchased from Micro labs limited, India. All the kits used for bioassay were purchased from Randox laboratories Ltd., Crumlin, Co. Antrim, UK. All chemicals and reagents were of analytical grades and the water used was double distilled.

2.2. Collection of plant

Fresh green leaves of *B. pinnatum* were obtained from a local farmland in Ijurin-Ekiti, Ekiti State, Nigeria. Identification and authentication of the plant was carried out at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria. A sample of the plant portion was deposited at the departmental herbarium with the specimen voucher number UHAE 2017/064.

2.3. Plant material

The leaves of the plant were shredded and air-dried at room temperature for a period of 30 days to remove water content. Dried leaves were blended to powder and kept in a sealed vial for further analysis.

2.4. Preparation of plant extracts

One hundred grams of the blended leave sample was soaked in distilled water for 24 h. It was then filtered using Whatman no. 4 filter paper. The filtrate was then concentrated using a rotary evaporator (Stuart, RE300/MS, Bibby Sterilin Ltd. UK) at 45 °C and dried by means of a freeze dryer. The extract at respective doses was administered orally using gavage.

2.5. Experimental animal studies

Healthy albino rats of Wistar strain (170 ± 20.24 g) used for this experiment were purchased from College of Medicine animal house, Afe Babalola University, Ado Ekiti. All animals were housed in cages in the animal house of the Department of Biochemistry, Afe Babalola University, Ado-Ekiti, at a temperature of 22 ± 3 °C on a 12-h light/dark cycle, with free access to commercially available standard pelleted feed and water *ad libitum*. All animals were acclimatized for 7 days prior to the commencement of the experiments. The procedures adopted in this study were in accordance with Guidelines for Care and Use of Laboratory Animals in Biomedical Research of the National Institutes of Health of the United States (NIH Publication, revised in 1985). All animal experiments were approved by the animal care committee of the Afe Babalola University Research Centre, Ado-Ekiti, Ekiti State, Nigeria, with protocol number ABUAD-SCI04/03/16/012.

2.6. Animal grouping

After a 7-day acclimatization period, the rats were randomly divided into six groups (n = 5) with free access to food and water.

- Group I rats served as control group.
- Group II animals received CCl_4 in olive oil (3 mg/kg body weight) *via* a single intraperitoneal (i.p) injection and served as toxicant group.
- Group III animals were pre-treated with silymarin (25 mg/kg body weight) orally for 14 days, followed by a single i.p injection of CCl_4.
- Group IV rats were pre-treated with AEBP leaves (25 mg/kg body weight) orally for 14 days, followed by a single i.p injection of CCl_4.
- Group V rats were pre-treated with AEBP leaves (50 mg/kg body weight) orally for 14 days, followed by a single i.p injection of CCl_4.
- Group VI animals received AEBP leaves only (50 mg/kg body weight) orally for 14 days.

2.7. Collection of blood and organs

Animals were euthanized 24 h after intoxication with CCl_4 by inhalation of anesthesia (chloroform), following an overnight fast. After loss of sensory, blood was rapidly withdrawn into a plain bottle by cardiac puncture. The blood samples were allowed to stand at room temperature for 30 min to clot, and then centrifuged at 3500 × *g* (800 D Desktop Electric Medical Lab. Centrifuge, China) for 10 min to obtain the serum, which was used for various analyses. Kidneys from animal were harvested, blotted free of adhering fluid and weighed. These were rinsed in cold 0.1 M Tris–HCl buffer (1:3, w/v pH 7.4) to remove blood stains, and subsequently homogenized in 0.1 M Tris–HCl buffer (1:5, w/v, pH 7.4) with about 10-up-and–down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenate was centrifuged at 4000 × *g*. The clear supernatants obtained were used for various biochemical assays.

2.8. Biochemical analyses and histopathological studies

Biochemical analyses were carried out in serum and tissue homogenate (kidney). The serum samples were used to assess the levels of renal markers (urea and creatinine) using commercially available kits from Randox Laboratories, UK. For the tissue renal marker, arginase II activity was evaluated using colorimetric method described by Hrabák et al. [24], while antioxidant key enzymes linked to the renal damage such as superoxide dismutase (SOD) and catalase (CAT) were determined using colorimetric method described by Alía et al. [25] and Sinha [26], respectively. Non-enzymatic antioxidant; total thiol (TSH) and non-protein thiol(NPSH) contents were determined using the method of Ellman [27]. Oxidative stress markers (nitric oxide (NO) and malondialdehyde (MDA) were determined by spectrophotometric method, as described by Morakinyo et al. [28] and Varshney and Kale [29], respectively. Histopathological examination of rats' kidney was done [30].