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Ethylacetate extract of red onion (*Allium cepa* L.) tunic affects hemodynamic parameters in rats

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Abstract

The effects of ethylacetate extract of red onion (*Allium cepa*) tunic (ACTE) on some hemodynamic and biochemical parameters were evaluated in normotensive albino rats. Blood pressure parameters were determined in anaesthetized rats orally administered ACTE (10-, 20-, or 40 mg/kg) or ramipril (1 mg/kg) once daily for 14 days. Respectively, 10-, 20-, or 40 mg/kg ACTE produced significant ($P < 0.01$), dose-dependent fall in systolic blood pressure, SBP (21%, 27%, 33%), diastolic blood pressure, DBP (6%, 10%, 16%), pulse pressure, PP (42%, 49%, 56%), mean arterial blood pressure, MAPB (13%, 18%, 23%) and heart rate, HR (4%, 5%, 7%). The highest effective dose (40 mg/kg) compared well with ramipril (1 mg/kg) with regards to SBP (41%), DBP (19%), PP (70%), MABP (29%) and HR (10%). Similar trends (decreases) were recorded for 40 mg/kg ACTE and ramipril, respectively, as regards the activities of serum enzymes: creatine kinase (60% and 65%), ALT (18% and 14%) and ALP (28% and 16%). HPLC fingerprints of the flavonoid-rich ACTE revealed that flavonols: quercetin, quercitrin, isoquercitrin, rutin and kaempferol are the active flavonoids. The results demonstrate the hypotensive effect of *A. cepa* tunic flavonoids initiating further investigation of their individual or synergistic contribution(s) to the observed effects.

Keywords: *Allium cepa*; Extract; Flavonoids; Hemodynamic; Hypotension

1. Introduction

Agents with hypotensive effect could be of therapeutic significance in the advent of clinical or experimental-induced hypertension. One quarter of the world's adult population is afflicted by hypertension, and this is likely to increase to 29% by 2025 [1]. The common progressive disorder leads to several chronic diseases such as cardiovascular disease, stroke, renal disease and diabetes [2].

Plants constitute a rich and diverse source of secondary metabolites that have long contributed to the development of small-molecule based therapeutics [3]. Onion (*Allium cepa* L.), a bulbous herb belonging to the family Alliceae, is a widely consumed vegetable. It is a good source of dietary phytochemicals including organosulphur compounds and flavonoids [4]. As a result of their phytoconstituents, onions exhibit considerable antioxidant properties and could modulate the detoxification systems [5]. Thus, onion intake is reported to have several beneficial effects on health, such as preventing tumors and cancers [6], cardiovascular diseases [7] and hypertension [8]. Most of the documented beneficial effects of onions,

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including inhibition of platelet aggregation [9], hypoglycaemic, hypolipidemic and antiatherosclerotic [10,11], and antioxidant and antiapoptotic effects [12], have centered on the bulb or other edible parts rather than the tunic which is normally discarded as wastes. We envisaged that this underutilized part of onion, which has not received much attention all the while, may have potential benefits to human health. To the best of our knowledge, there is a dearth of information on effect of red onion (*A. cepa*) tunic on hemodynamic parameters. This work was therefore initiated to provide scientific information in this regard.

2. Materials and methods

2.1. Chemicals

Glutathione, 5′,5′-dithiobis-(2-nitrobenzoate) DTNB, epinephrine and hydrogen peroxide were purchased from Sigma Chem., Co. (London, UK). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), total protein and total cholesterol kits were obtained from Randox Laboratories, UK. All other chemicals were of analytical grade and were either obtained from Sigma–Aldrich or British Drug Houses, (Poole, UK).

2.2. Plant material

Red onions (*A. cepa*) were bought from Shasha market, outskirt Akure metropolis, Nigeria, in the month of February 2013. Botanical identification and authentication were carried out at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria.

2.3. Preparation of ethylacetate extract of red onions (*A. cepa*) tunic

The tunics of onions were removed, cleaned, air-dried and pulverized. The powdered sample (80 g) was macerated in 600 mL of ethyl acetate (Sigma Chemicals; USA) for 4 h at 40 °C, with constant agitation. The mixture of the solvent and the ground sample was filtered first with mesh cloth, and then with filter paper (Whatman No. 1) and concentrated using a rotary evaporator. The residue was kept in a refrigerator at 4 °C until further uses. Prior to the experiments, the ethylacetate extract was dissolved in distilled water and diluted to the desired concentrations to give a water-soluble fraction (ACTE).

2.4. Phytochemical screening

ACTE was screened for the presence of alkaloids, saponins, tannins, steroids, anthraquinones, terpenoids, flavonoids and phlobatannins as described in our previous work [13].

2.5. Determination of total flavonoid content

The colorimetric method described by Dewanto et al. [14] was employed in the quantification of total flavonoids in ACTE. To 250 μL of the suitably diluted sample, 75 μL of 5% $NaNO_2$ solution, 150 μL of freshly prepared 10% $AlCl_3$ solution, and 500 μL of 1 mol/L NaOH solution were added. The final volume was adjusted to 2.5 mL with deionized water and the mixture left to stand for 5 min. The absorption was thereafter measured at 510 nm against the same mixture, without the sample, as blank. The amount of total flavonoids was expressed as quercetin equivalents (QE, mg quercetin/g sample) through the calibration curve of quercetin.

2.6. Quantification of flavonoids

Reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm × 150 mm) packed with 5 μm diameter particles; the mobile phase was water containing 2% acetic acid (A) and methanol (B), and the composition gradient was: 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70% and 100% B at 10 min, 20 min, 30 min, 40 min, 50 min and 60 min, respectively, following the method described by Peroza et al. [15] with slight modifications. ACTE was analyzed at a concentration of 12 mg/mL. The presence of flavonoids was investigated, namely, quercetin, isoquercitrin, quercitrin, rutin and kaempferol. Identification of these compounds was performed by comparing their retention time and UV absorption spectra with those of commercially available standards and by DAD spectra (300–600 nm). The flow rate was 0.7 mL/min, injection volume 50 μL and the wavelength was 356 nm. The samples and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.050–0.500 mg/mL for quercetin, isoquercitrin, quercitrin, kaempferol and rutin. All chromatography operations were carried out at ambient temperature and in triplicate.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Sabir et al. [16]. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.7. Animals

Adult male rats (Wistar strain) weighing 200–220 g, obtained from a private breeder and housed in the primate colony of the Department of Physiology, College of Medicine, University of Lagos, Nigeria were used for this study. The animals were kept in wire mesh cages under controlled light cycle (12 h light/12 h dark), fed with commercial rat chow (Vital Feeds Nigeria Limited) ad libitum, and liberally supplied with water. All animal experiments were conducted according to the guidelines of National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use.