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Exploration of antioxidant, antidiabetic and hepatoprotective activity of *Diplazium esculentum* - A wild edible plant from North Eastern India

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ABSTRACT

Objective: The present work is undertaken for phytochemical and pharmacological investigation of *Diplazium esculentum* leaf's hydroalcoholic extract (DEHAe).
Methods: Antioxidant activity was estimated by DPPH assay and nitric oxide (NO) inhibition was determined using Griess reagent. Diabetes was induced artificially by streptozotocin (STZ). DEHAe was administered orally to see its affect on fasting glucose level, lipid profile, biochemical parameters and antioxidant enzyme activity. Hepatoprotective activity was examined by carbon tetrachloride (CCl_4) induced hepatic injury method. Histological slides of pancreas were prepared to observe the histological changes.
Results: The experiments yielded expected activity of the plant extract in animal model. IC_{50} value of DEHAe for *in vitro* antioxidant activity and NO inhibition activity was found to be 138.8 and 151.9 μg/mL respectively. Fasting blood glucose level was reduced 50.2% by DEHAe (500 mg/kg) in STZ induced diabetic rats. Significant normalization ($p < 0.01$ and $p < 0.05$) of the lipid profile and serum marker enzymes were observed in the diabetic rats treated with DEHAe. Sufficient normalization of antioxidant enzyme activity was observed by DEHAe administration. CCl_4 had a little impact on the liver enzyme activity and total biluribin on the rats pre-treated with DEHAe. Pancreas of DEHAe treated animals showed a significant reduction of necrosis and regeneration of β-cells.
Conclusion: The present experiment shows that DEHAe has significant activity on free radical scavenging, antidiabetic, hyperlipidimic and hepatoprotective activity and thus can be consumed as a functional vegetable.

1. Introduction

The increasing physiological disorder and degenerative diseases attributed by the food habit and environmental pollution, there is a considerable increase in research in natural products especially edible plants to formulate balanced and functional diet. Despite of taking supplements it is more convenience and effective to take a well formulated diet. The pharmaceutical sciences also striving to substitute the therapeutic medicines and formulations with plant derived product for better efficacy and least adverse affect. The development of a new drug or a therapeutic medicine starts with the baseline screening of the individual plants from different perspectives. Intensive search for novel antioxidant agents is being undertaken in numerous plants for the management of the disease like diabetes [1]. World health Organization (WHO) is giving prime importance in the evaluation of the natural products for the treatment of diabetes for their less toxicity and board spectrum management due to the presence of various biomolecules.

Diabetes mellitus (DM) is a chronic carbohydrate, fat and protein metabolism disorder characterized by the increased in blood glucose level due to defect of insulin secretion or insulin action [2]. Although DM is not a communicable disease, it is the

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fifth major cause of death worldwide affecting 150 million populations and predicted to rise to 300 million by 2025 if the same trend used to prevail unchecked. The alarming rate of increase in diabetic patient and highest diabetic subjects in India has earned itself the dubious distinction term by 'Diabetes Capital of the World' [3].

Reduction of the antioxidant activity coexists with oxidative stress in diabetes which eventually increases the deleterious effect of the free radicals [1]. The excess blood glucose produces superoxide anions and where Haber-Weiss reaction takes place producing hydroxyl radical which results in protein glycation and peroxidation of membrane lipids which greatly damages the biomolecules including deoxyribonucleic acid [4]. Antioxidants prevent the free radical mediated damages by scavenging them and thereby protecting from oxidative stress. In recent days more emphasis is being paid to develop drug having both the antidiabetic and antioxidant activity to effectively treat DM [3,4].

DE (Retz.) Sw. (Athyriaceae) is a wild edible rhizomatous fern mainly consumed as vegetables which are probably the most consumed fern along the hill tribe of North Eastern India and Western Ghats [5,6]. The young fronds are used in myriad of way to prepare local dishes including stir-fried and salads. The fern is believed to contain various medicinal properties and some of them are evaluated and confirmed by research. It act as mast cell stabilizer and can prevent anaphylactic shock [7], decoction of the plant can be used to treat hemoptysis and cough [8]. The plant is also reported to use traditionally for the treatment of dysentery, glandular swellings, indigestion, diarrhea and various skin infections [9]. The present study is carried out to investigate its antidiabetic activity together with *in vitro* and *in vivo* antioxidant activity, hyperlipidimia and hepatoprotective activity.

2. Materials and methods

2.1. Chemicals

STZ was purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India), CCl_4 from Fisher Scientific UK Ltd, DPPH-1, 1-diphenyl- 2-picryl-hydrazyl from Sigma-Aldrich, St. Louis, USA. Silymarin was obtained from Quality Pharma Products Pvt. Ltd. Dibrugarh (India). All the other chemicals and reagents used in the experiments were of analytical grade.

2.2. Plant collection and extract preparation

Leaves of DE were collected on 28-06-2014 from Dibrugarh district of Assam, India. The plant was identified and authenticated by Dr A. A Mao, Scientist E, BSI, Eastern Regional Centre, Shillong, Meghalaya (BSI/ERC/2014/Plant identification/360 dated 26-08-2014) and voucher specimen (DU/PSC/HRB-10/2014) was deposited there for further reference. The leaves were shade dried at ambient temperature until completely dehydrated. The dried materials were grounded using electric grinder to produce coarse powder and kept in vacuum desiccators until solvent extraction. The powdered material (200 g) was macerated overnight with hydroalcoholic solution. The sample was then extracted several times till the coloured materials ceases to precipitate with the solvent. DE leaves hydroalcoholic extract (DEHAe) was filtered and excess solvent were removed in rotary vacuum evaporator and concentrated by freeze drying. The crude extract was then kept at −20 °C prior to analysis. The extractive value of DEHAe was found to be 17.46% w/w.

2.3. Phytochemical analysis

The presence of various group of phytoconstituents in DEHAe were screened using various standard photochemical detection tests as described earlier with modifications wherever necessary [10].

2.4. Total phenolics

The total phenolic of the hydroalcoholic extracted sample was determined calorimetrically using Folin-Ciocalteu assay [11]. An aliquot of 1 mL of sample was mixed with 1 mL of Folin-Ciocalteu reagent (FCR). After 3 min, 1 mL of saturated sodium carbonate (Na_2CO_3) solution was added to the mixture and the volume was adjusted to 10 mL with distilled water. The reaction mixture was kept in dark for 90 min, after which the absorbance was taken at $\lambda = 725$ nm against the blank. Standard calibration curve was drawn using Gallic acid (50–250 mg/mL) against its absorbance and the result was expressed as Gallic acid equivalent (GAE) per mL of the test solution.

2.5. Total flavonoids

The total flavonoids were determined calorimetrically by aluminum chloride method described by Chang et al. (2002) [12]. Hydroalcoholic solution (0.5 mL) was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. The reaction mixture was incubated in dark at room temperature for 30 min and then the wavelength was set at $\lambda = 415$ nm against the blank sample prepared by substituting aluminum chloride with the same volume of distilled water. The calibration curve was constructed with quercetin of different concentration (50–250 mg/mL) against its absorbance at $\lambda = 415$ nm.

2.6. DPPH antioxidant assay

The free radical scavenging activity of all extracts were determined by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) antioxidant assay according to method reported by Shen et al. (2010) [13] with slight modification. Briefly, a 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of the hydroalcoholic extract solution in methanol at different concentration (50,100, 150, 200, 250 μg/mL). The mixtures were shaken vigorously and allowed to stand at dark in room temperature for 30 min, then the absorbance was measured at $\lambda = 517$ nm under dim light using a UV-VIS spectrophotometer (UV-1700 PharmaSpec, SHIMADZU). Deionised water was used for blank reading and ascorbic acid was used as standard solution. The percentage of scavenging activity was calculated by the following equation.

$$\text{DPPH Scavenged}(\%) = \left[\frac{Absorption\ Control_{517} - Absorption\ Sample_{517}}{Absorption\ Control_{517}}\right] \times 100$$