SUPPLEMENTARY MATERIAL

Biofunctional properties of *Eruca sativa* Miller (rocket salad) hydroalcoholic extract.

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Abstract

Eruca sativa Miller is a worldwide common alimentary plant (rocket salad). Besides glucosinolates, it is also an excellent source of fibers, minerals and secondary metabolites such as ascorbic acid, flavonoids and carotenoids which are known as antioxidants molecules able to scavenge free radicals. The aim of this study was to correlate the potential *in vitro* scavenging activity of the *E. sativa* hydroalcoholic extract (HAE) with its *in vivo* hypoglycemic effect. In DDPH free radical (DFR) and ferric-reducing antioxidant power (FRAP) assays, HAE in a concentration dependent manner (25-100 µg/mL) displayed a strong scavenging activity with maximum effect of 88% and 75% at 100 µg/mL, respectively. On *in vivo* model of alloxan-induced diabetic rabbits, the daily administration of HAE (50 mg/kg; p.o.) for 28 days showed a significant reduction of glycemia, also supported by a recovery of body weight. In conclusion, our results give preliminary information on the potential use of this plant as a nutraceutical, useful to control and/or prevent a hyperglycemic status.

Keywords: *Eruca sativa* Miller, rocket salad, hypoglycemic activity, antioxidant.

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Experimental

Plant materials

For seasonal experiments, *E. sativa* fresh leaves and stems were collected at commercial maturity (when leaves were 10-13 cm in length). Taxonomic identity was determinate at Department of Plant Sciences, Kohat University of Science and Technology, Kohat, Pakistan. A voucher specimen (No. 20040) was deposited at Herbarium of the same Department.

Extraction procedure

Coarsely powdered leaves and stems of *E. sativa* (3 kg) were subjected to maceration at room temperature for 7 days with methanol/water (50:50, v/v). Alcohol used for the extraction procedure was removed on a rotary evaporator and under pressure at 42°C. The residue was heated in a water bath at 40°C for further thickening to yield the crude extract (150 g). The residues were successively collected, weighted and diluted in 0.2M phosphate buffer (pH:6.6) for the in vitro experiments and dissolved in saline for the in vivo procedures. The extraction process described above was repeated several times in order to obtain an adequate amount of the extract for carrying out the *in vitro/vivo* experiments.

Animals

Healthy locally breed rabbits (1.2-1.5 kg) were used for all the in vivo experiments. The animals were maintained in standard conditions ($22 \pm 2^\circ\text{C}$ and light/dark cycles i.e. 12/12 h) and were fed with standard diet and water *ad libitum*. The experimental protocols were approved by the ethical Committee of the Department of Zoology, University of Science and Technology (Kohat, Pakistan). All efforts were made to minimize animal suffering by treating them according to international standards of animal use for experimental purposes.

DPPH radical scavenging assay

The antioxidant activity of HAE was performed by DPPH radical scavenging assay. The electron donation abilities of the corresponding compounds and standards drug (quercetrine) were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). All Analysis was carried out in triplicate according to the standard procedure (Khan et al., 2013). Briefly, a 1 mM solution of DPPH radical solution in methanol was prepared and 1mL of this solution was mixed with 3 mL of sample solutions in methanol (containing 5-100 µg/mL) and control (without sample). The solution was stand for 30 min, in

dark the absorbance value was monitored by using spectrophotometer at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follows

$$\% \text{ DPPH} = \frac{(\text{OD Control} - \text{OD sample})}{\text{OD control}} \times 100$$

Where, OD control is the absorbance of the blank sample, and OD sample is the absorbance of samples or standard sample. IC₅₀ was the determined as 50% scavenging of test compounds on DPPH.

Ferric-reducing antioxidant power (FRAP) assay

In vitro, the reducing power of HAE was performed as previously described (Patel et al., 2010) and tested in a concentration range of 25-100 µg/mL in 0.2M phosphate buffer (pH:6.6) containing 1% ferricyanide. The mixture was incubated at 50 °C for 20 minutes. Successively, 2.5 mL of 10% trichloroacetic acid (TCA) was added to this mixture (5 mL) and centrifuged at 3,000 g for 10 minutes. The supernatant was separated and mixed with distilled water (2.5 mL) containing 1% ferric chloride (0.5 mL). The procedure was repeated in triplicate and allowed to stand for 30 min before measuring the absorbance at 593 nm. The FRAP assay has been validated using butylated hydroxytoluene (BHT) as standard antioxidant (Ahmed et al., 2010). The percentage of antioxidant activity in the assay was calculated according to the formula: Scavenging activity (%) = (A1 x A0) / A1. Where, A0 = Absorbance of the control (potassium phosphate buffer + FRAP reagent). A1 = Absorbance of sample.

Alloxan-induced hyperglycemic rabbits and growth performance of rabbits body weight

For the induction of this experimental model, the animals were made hyperglycemic by means of alloxan monohydrate. It was administered by i.v ear vein at the dose rate of 150 mg/kg as previously described (Vogel et al., 2002). Rabbits with a blood glucose level ≥140 mg/dL were considered as hyperglycemic (for a maximum of selected values of 160 mg/dL). Animals with a blood glucose levels ≤ 80 mg/dL were discarded from the experiments. Blood samples were collected from ear vein and recorded by the mean of a glucometer (Accu-Chek, Active, Roach, Pakistan) after 1,2, 7,14, 21 and 28 days after alloxan administration (Ahmed et al., 2010). Rabbits total weight was recorded before alloxan injection (initial body weight) and after treatments (final body weight).

Experimental design

Twenty four healthy rabbits were divided in four groups (n=6). All the oral treatments (p.o.) were performed daily by the aim of a gastric gavage. Group I: hyperglycemic rabbits; Group II: *Eruca sativa* hydroalcoholic extract (HAE; 50 mg/kg, p.o.); Group III: glibenclimide (GLB; 5 mg/kg, p.o.); Group IV: normoglycemic rabbits (saline; 10 mL/kg).

Statistical analysis

The results obtained were expressed as the mean $\pm$ S.E.M. For statistical analysis, ANOVA was followed by post hoc Dunnett's test for multiple comparisons. The statistical analysis was carried out by GraphPad PRISM 6 (San Diego, CA, USA). Values were considered to be significant at $P < 0.05$ (* $P < 0.05$; ** $P < 0.01$).

Figure S1. Effect of HAE (25-100 $\mu\text{g/mL}$) on DDPH free radical assay. The results obtained were expressed as the mean $\pm$ S.E.M. of the percentage (%) of scavenging activity. * $P \leq 0.05$, ** $P \leq 0.01$ compared to control ($n=3$ of three different experiments).

Figure S2. Effect of HAE (25-100 µg/mL) on ferric-reducing antioxidant power (FRAP) assay.

The results obtained were expressed as the mean $\pm$ S.E.M. of the percentage (%) of scavenging activity. * $P \leq 0.05$, ** $P \leq 0.01$ compared to control ($n=3$ of three different experiments).

Figure S3. Effect of HAE (50 mg/kg, p.o.); glibenclamide (GLB; 5 mg/kg, p.o.) on blood glucose level in hyperglycemic rabbits during a drug trial of 28 days. Values are expressed as mean $\pm$ S.E.M. * $P \leq 0.05$, ** $P \leq 0.01$ compared to hyperglycemic rabbits (n=6 for each group).

References

- Ahmed MF, Kazim SM, Ghorri SS, Mehjabeen SS, Ahmed SR, Ali SM, Ibrahim M. 2010. Antidiabetic Activity of *Vinca rosea* Extracts in Alloxan-Induced Diabetic Rats. *Int J Endocrinol*. doi: 10.1155/2010/841090.
- Khan H, Saeed M, Muhammad N, Rauf A, Khan AZ, Ullah R. 2013. Antioxidant profile of constituents isolated from *Polygonatum verticillatum* rhizomes. *Toxicol Indus Health*:0748233713498454.
- Patel A, Amit Patel A, Patel A, Patel NM. 2010. Determination of polyphenols and free radical scavenging activity of *Tephrosia purpurea* linn leaves (Leguminosae). *Pharmacognosy Res*. 3: 152–158.
- Vogel HG. 2002. Methods to induce experimental diabetes mellitus. In *Drug discovery and evaluation*. Vogel HG (Ed), Springer-Verlag, Berlin, Heidelberg, New York, 950-951.