Journal of Medicinal Plants Research Vol. 7(2), pp. 85-90, 10 January, 2013
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.915
ISSN 1996-0875 ©2013 Academic Journals

Full Length Research Paper

In vitro and *in vivo* antioxidant properties of Tunisian carob (*Ceratonia siliqua* L.)

Hichem Sebai[1,2,*#], Abdelaziz Souli[2,3#], Latifa Chehimi[1], Kais Rtibi[2,3], Mohamed Amri[3], Jamel El-Benna[4] and Mohsen Sakly[1]

[1]Laboratoire de Physiologie Intégrée, Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisia.
[2]Laboratoire de Nutrition et Physiologie Animale - Institut Supérieur de Biotechnologie de Béja, Avenue Habib Bourguiba - B. P. 382 - 9000 Béja, Tunisia.
[3]Laboratoire de Neurophysiologie Fonctionnelle et Pathologies, Département des Sciences Biologiques, Faculté des Sciences de Tunis. Campus Universitaire El Manar II-2092 Tunis, Tunisia.
[4]INSERM U773 Centre de Recherche Biomédicale - Faculté de Médecine X. Bichat 75018 - Paris France.

Accepted 15 August, 2012

The present study aims at the quantification of phenolic compounds of Tunisian carob (*Ceratonia siliqua* L.) pods and the study of their *in vitro* and *in vivo* antioxidant activities in different extraction solvents. In this respect, we used 50 rats divided into five groups: Control and Carob-treated groups (100, 200, 600 and 1000 mg/kg, *b.w.*). Our results showed that the carob polar extracts are richer in total polyphenols, total flavonoids and condensed tannins than the nonpolar extracts with quantitative variation of phenolic compounds between seeds and pulp. *In vitro*, the determination of antioxidant capacity by 2,2'-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid] (ABTS) method showed that carob extracts present high antioxidant potency in a solvent-compartment-dependent way compared to Trolox, an antioxidant reference molecule. *In vivo*, the subacute treatment within 7 days with aqueous extract of carob pods (AECP) decreased significantly and doses dependently the cerebral and myocardial lipid peroxidation as well as the level of hydrogen peroxide (H_2O_2) in kidney, liver and brain but not in heart. These data suggest that carob fruit presents an *in vitro* an *in vivo* antioxidant effects and might be proposed as a food additive to protect against oxidative stress damage.

Key words: Carob, phenolic compounds, antioxidant properties, rat.

INTRODUCTION

Several years scientists are interested in the reactive oxygen species (ROS) that are implicated in many human diseases (Lobo et al., 2010). Increased ROS lead to oxidative stress and a degenerative signalling cascade triggered by oxidation of vital cellular components, which induced cellular damage and cell death (Farrugia and Balzan, 2012).

Oxidative stress status is characterized by depletion from intracellular stores of endogenous antioxidants or by rapid alteration in antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), resulting in increased lipoperoxidation (Ikeda et al., 2004). To protect against these harmful species we use a synthetic or natural antioxidants molecules (Jacobo-Velázquez et al., 2009; Nabavi et al., 2012) which are able to scavenge ROS and to up-regulate endogenous antioxidant defences (Migdal and Serres, 2011).

The carob tree (*Ceratonia siliqua* L. Leguminosae

*Corresponding author. E-mail: sebaihichem@yahoo.fr. Tel: +(216) 97 249 486. Fax: +(216) 72 590 566.

Abbreviations: **ABTS,** 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); **AECP,** aqueous extract of carob pods; **CAT,** catalase; **GPx,** glutathione peroxidase; **MDA,** malondialdehyde; **ROS,** reactive oxygen species; **SOD,** superoxide dismutase; **TEAC,** trolox equivalent antioxidant capacity.

#This two first author's contributed equally to this work.

family) has been widely cultivated for years in Mediterranean countries including Tunisia (Rejeb, 1995). The carob fruit, brown pod 10 to 25 cm in length, contain two major components; the pulp and the seeds (Turnbull et al., 2006). The important ingredient in the seeds is galactomannan which is known for thickening effects and widely used in food industry (Marakis, 1996). The carob pods are used for the production of animal feed or in human foods as a substitute for cocoa (Kumazawa et al., 2002). However, carob pods contain a large amount of condensed tannins (Bravo et al., 1994) compared to leaves (Silanikove et al., 2001). Importantly, carob extracts have several beneficial effects on health such us cholesterol lowering activities in humans suffering from hypercholesterolemia (Zunft et al., 2001, 2003) and antioxidant properties in different *in vitro* test systems (Custodio et al., 2011). Recent studies discovered that Tunisian leaf carob extract presents some ameliorative effects against CCl_4-induced oxidative damage in rats tissues (Hsouna et al., 2011).

The present study aimed to investigate the *in vitro* and *in vivo* antioxidant properties of carob pods by the determination of phenolic compounds content and antioxidant capacity in different extraction solvents as well as the effects of AECP on tissue lipid peroxidation and H_2O_2 levels in healthy rats.

MATERIALS AND METHODS

Chemicals

2-Thio-barbituric acid (TBA), Trolox, Folin-Ciocalteu reagent, gallic acid, vanillin, catechin and quercetin were from Sigma chemicals Co (Germany). All other chemicals were of analytical grade.

Preparation of carob extract for *in vitro* and *in vivo* examinations

Immature carob pods (*Ceratonia siliqua* L.) were cultivated from the region of Tabarka (NW-Tunisia) during june-2011. Pulp and seeds were separately dried in an incubator at 50°C during 48 h and powdered in an electric blender. Powders were extracted with water, methanol, ethanol, acetone, ether petroleum and hexane at room temperature and centrifuged 10 min at 10 000 g to eliminate insoluble materials. The extracts were evaporated to dryness *in vacuo*, weighed and stored at -80°C until use. For *in vivo* examination, powder mixture containing carob pulp (90%) and seeds (10%) was used and followed the same route of extraction.

Animals and treatment

Adult male Wistar rats (weighing 220 to 240 g and housed five per cage) were purchased from Pasteur Institute of Tunis and used in accordance with the local ethic committee of Tunis University for use and care of animals in conformity with the NIH recommendations. They were provided with standard food (ALMES, TN) and water *ad libitum* and maintained in animal house at controlled temperature (22 ± 2°C) with a 12 h light-dark cycle. Rats were divided into 5 groups of 10 animals each: Control and Carob-treated groups (100, 200, 600 and 1000 mg/kg, *b. w.*). Animals were daily intraperitoneally (*i. p.*) injected during 7 days with vehicle (control, bidistilled water) or various doses of AECP (injection volume was 1 mL/kg *b.w.*). Twenty four hours after the last carob injection animals were sacrificed, their Brain, liver, kidney and heart were rapidly excised and homogenized in phosphate buffer saline pH 7.4. After centrifugation at 10000 g for 10 min at 4°C, supernatant was used for biochemical determination of protein, MDA and H_2O_2.

Total polyphenols determination

The determination of total polyphenols was conducted according to the method of Haseeb et al. (2006). Briefly, in alkaline medium, polyphenols reduce phosphomolybdic acid of Folin Ciocalteu, this reduction leads to the appearance of a blue color measured at 765 nm. Gallic acid was used as standard.

Total flavonoids determination

The content of total flavonoids was performed according to (Bahorun et al., 1996). Briefly, the flavonoids have a free hydroxyl group (OH^-), in position 5 which is capable of reacting with the CO group and giving a colored complex with aluminum chloride. Flavonoids form by chelation of aluminum a yellow complex measured at 430 nm. Quercetin was used as standard.

Condensed tannins determination

Condensed tannins are determined by the method of vanillin in acidic medium (Price et al., 1978). This method is based on the ability of vanillin to react with condensed tannin units in the presence of acid to produce a colored complex measured at 500 nm. Catechin was used as standard.

Antioxidant assay

The antioxidant capacities of organic solvent- and water-soluble fractions were evaluated using the Trolox equivalent antioxidant capacity (TEAC) method (Re et al., 1998), which measured the ability of a compound to scavenge the 2,2'-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid] (ABTS) radical.

Lipid peroxidation measurement

Tissue lipid peroxidation was determined by MDA measurement according to the double heating method (Draper and Hadley, 1990). Briefly, aliquots from tissue homogenates were mixed with BHT-TCA solution containing 1% BHT (w/v) dissolved in 20% TCA (w/v) and centrifuged at 1000 g for 5 min at 4°C. Supernatant was blended with 0.5 N HCl 120 mM TBA in 26 mM Tris and then heated at 80°C for 10 min. After cooling, absorbance of the resulting chromophore was determined at 532 nm using a UV-visible spectrophotometer (Beckman DU 640B). MDA levels were determined by using an extinction coefficient for MDA-TBA complex of $1.56\ 10^5\ M^{-1}cm^{-1}$.

H_2O_2 determination

The tissue H_2O_2 level was performed according to Dingeon et al. (1975). Briefly, in the presence of peroxidase, the hydrogen peroxide reacts with p-hydroxybenzoic acid and 4-aminoantipyrine leading to a quantitative formation of a quinoneimine which has a

Table 1. The content in total polyphenols, total flavonoids and condensed tannins for different tested carob extracts.

Compound (mg/kg)	Pulp seed	Water	Methanol	Ethanol	Acetone	Ether petroleum	Hexane
Total polyphenols	Pulp	28.07 ± 0.99	8.36 ± 0.48	22.27 ± 0.80	1.27 ± 0.15	0.27 ± 0.04	2.58 ± 0.27
	Seeds	18.16 ± 1.01	6.79 ± 0.04	13.16 ± 0.04	2.41 ± 0.17	0.48 ± 0.09	4.66 ± 0.18
Total flavonoids	Pulp	6.14 ± 1.03	2.49 ± 0.28	6.78 ± 0.94	0.68 ± 0.09	0.12 ± 0.03	1.58 ± 0.14
	Seeds	8.63 ± 1.27	3.66 ± 0.35	5.81 ± 0.43	1.2 ± 0.18	0.19 ± 0.05	2.83 ± 0.23
Condensed tannins	Pulp	6.71 ± 0.71	1.71 ± 0.21	3.49 ± 0.64	0.16 ± 0.03	0.05 ± 0.01	0.28 ± 0.08
	Seeds	6.97 ± 0.91	2.34 ± 0.94	6.59 ± 1.06	0.53 ± 0.12	0.08 ± 0.02	0.63 ± 0.11

Table 2. The percentage of ABTS reduction for different tested carob extracts.

Extract		Pulp (% inhibition)	Seeds (% inhibition)
Carob	Water	40.03	13.45
	Methanol	23.68	64.26
	Ethanol	10.32	14.42
	Acetone	9.04	3.21
	Ether petroleum	0.19	0.66
	Hexane	6.32	1.76
Reference solution	Trolox	79.41	

pink color detected at 505 nm.

Protein determination

Protein concentration was determined according to Hartree (1972) which is a slight modification of the Lowry method. Serum albumin was used as standard.

Statistical analysis

Data were analyzed by unpaired Student's *t*-test or one-way analysis of variance (ANOVA) and are expressed as means ± standard error of the mean (SEM). Data are representative of ten independent experiments. All statistical tests were two-tailed, and a *p* value of 0.05 or less was considered significant.

RESULTS

Determination of total polyphenols, total flavonoids and condensed tannins from carob pulp and seeds using various solvent systems

Data from Table 1 showed a higher rate of total polyphenols in the aqueous, methanol and ethanol extracts compared to other organic solvents used (acetone, ether petroleum and hexane) with a higher concentration in the pulp relative to the seeds. The same diagram of solubility was observed for total flavonoïdes with more concentration in the seeds. In contrast, the condensed tannins are more present in aqueous, methanol and ethanol extracts compared to acetone, ether petroleum and hexane extracts, with a higher concentration in the seeds.

Determination of pulp and seeds antioxidant potency

We also studied the variation of antioxidant power between the pulp and seeds in different extraction solvents (Table 2). Our result indicated a higher rate in scavenging the ABTS radical. The highest inhibition was observed for pulp in water extract and for seeds in methanol extract (40.03 and 64.26%, respectively) compared to 79.41% of inhibition for trolox, a well-known antioxidant molecule.

Effects of aqueous extract on tissue lipid peroxidation and H_2O_2 content

To test the *in vivo* effect of carob on tissue lipoperoxidation and H_2O_2 content, rats were treated with AECP for 7 consecutive days. The subacute treatment with various doses of AECP significantly and dose dependently decreased the cerebral and myocardial lipid peroxidation. However, the same treatment did not affect the hepatic and renal levels of MDA (Figure 1). ACPE administration also indicated a significant dose-

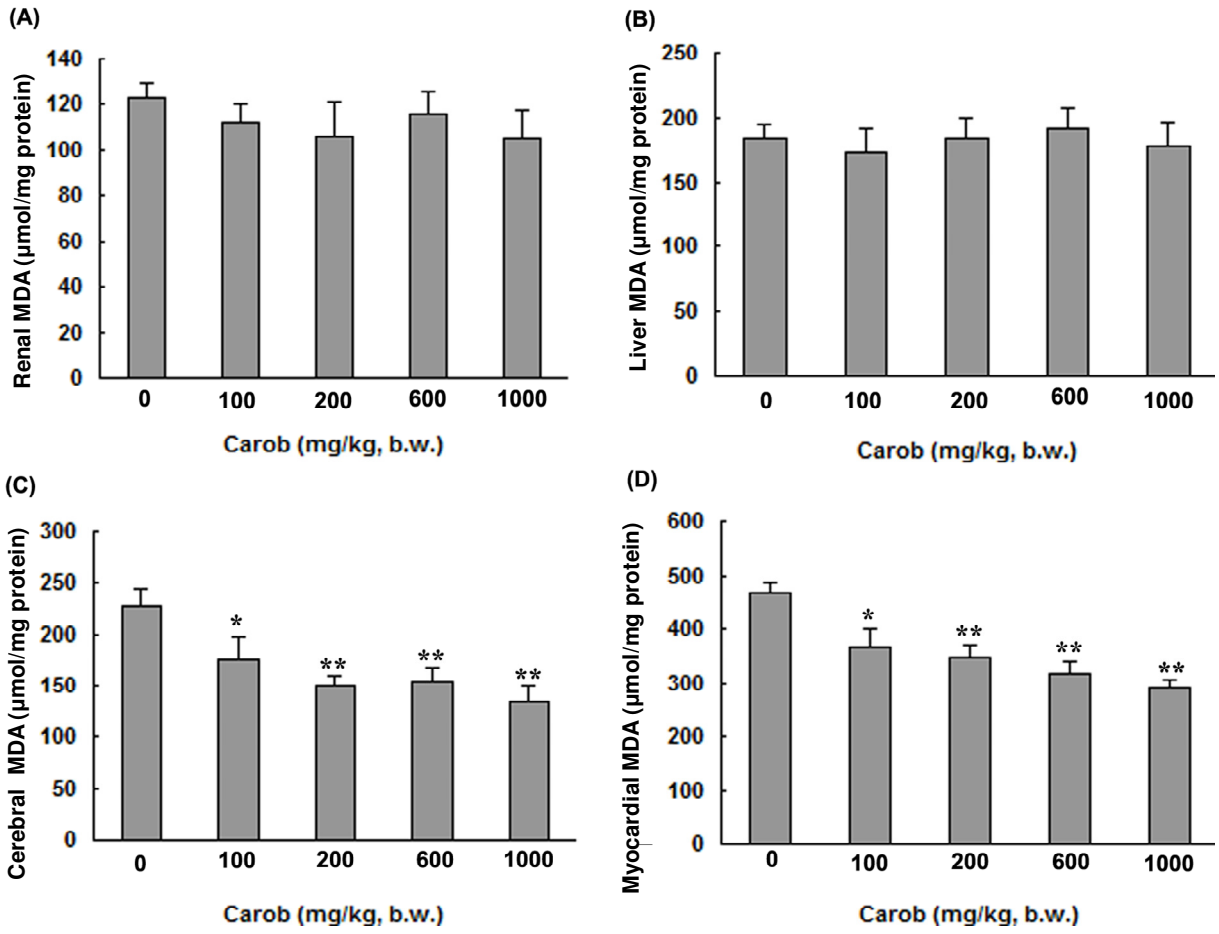

Figure 1. Dose-response effect of aqueous extract of carob pods (AECP) on kidney (A), liver (B), brain (C) and heart (D) MDA levels. Rats were treated during 7 days with indicated doses of AECP or vehicle (bidistilled H_2O). Assays were carried out in triplicate. Data are expressed by Mean ± SEM for n = 10 rats *indicated $P < 0.05$ and ** $P < 0.01$ *versus* control group.

dependently decrease of hydrogen peroxide in kidney, liver and brain but not in heart even with the highest dose (Figure 2).

DISCUSSION

We studied in the present paper the antioxidant properties of carob pulp and seeds by the quantification of phenolic compounds in different solvent extracts as well as the evaluation of their antioxidant capacity. We also examined the effects of selected fraction (AECP) on tissues lipid peroxidation and H_2O_2 contents in healthy rats.

We first found that the total polyphenols content was higher in the water fraction (28.07 mg/kg) compared to other organic solvents in the pulp relative to the seeds. The quantity of total flavonoids was also higher in aqueous and ethanol fractions in pulp (6.14 and 6.78 mg/kg, respectively) and also in seeds (8.63 and 5.81 mg/kg, respectively). The condensed tannins were elevated in the aqueous and ethanol fractions with a larger concentration in the seeds. Previous study indicated that carob pods contain 19.2% of total polyphenols and 4.37% of total flavonols (Kumazawa et al., 2002). Another examination of carobs showed 1.9 mg/g of total polyphenols, 0.28 mg/g of proanthocyanidins, and 0.1 mg/kg of hydrolysable tannins (Avallone et al., 1997). However, when other organic solvents as acetone (80%) were used, the levels of total polyphenols and total flavonols are not very far respectively 9.28 and 1% (Makris and Kefalas, 2004). To our knowledge we are the first who quantified phenolic compounds with different extraction solvents and in both compartments (pulp and seeds) simultaneously. However, this variability in polyphenols content is mainly related to the climatic conditions as well as the mode of extraction (Papagiannopoulos et al., 2004). Indeed, the slight modifications in the extracting medium may have a prominent impact on the amount and nature of the compounds recovered and therefore particular emphasis should be given to the selection of solvent system

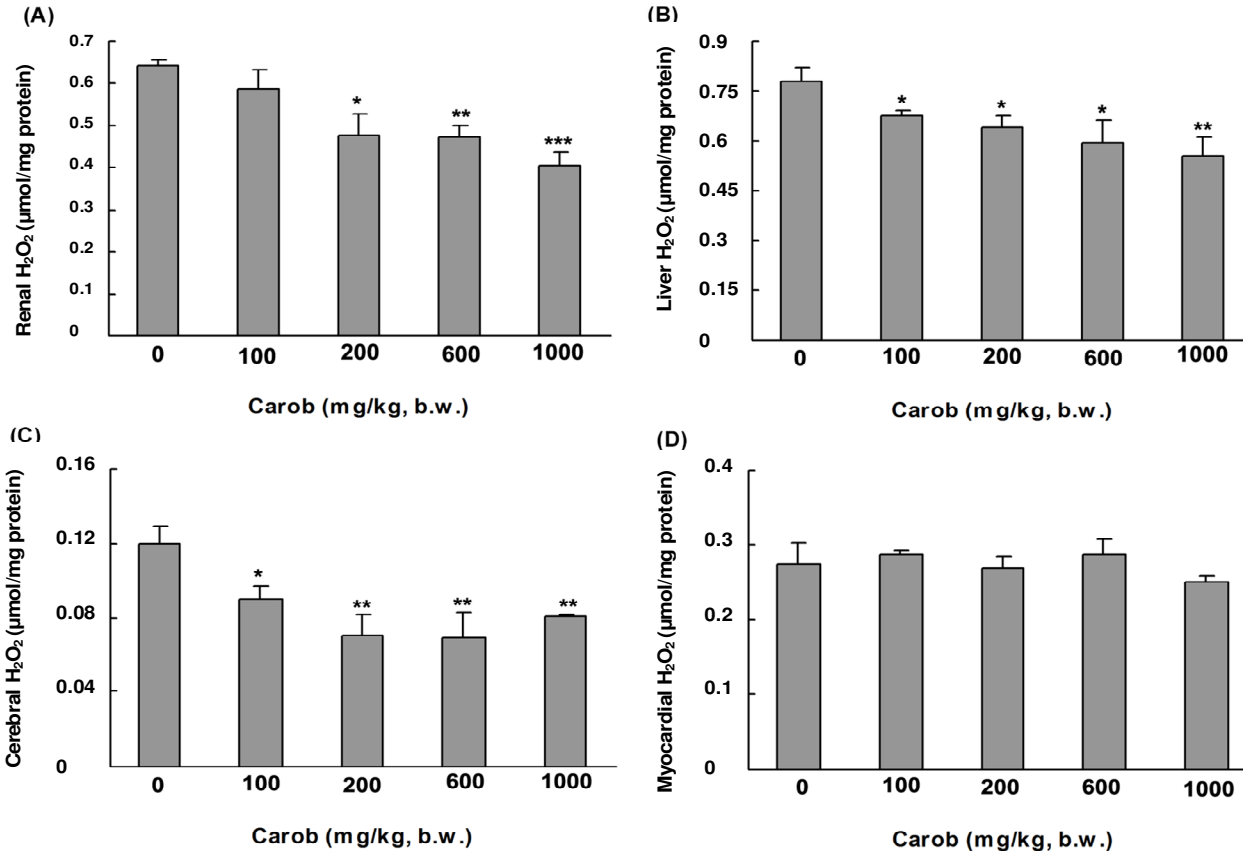

Figure 2. Dose–response effect of aqueous extract of carob pods (AECP) on kidney (**A**), liver (**B**), brain (**C**) and heart (**D**) H_2O_2 levels. Rats were treated during 7 days with indicated doses of AECP or vehicle (bidistilled H_2O). Assays were carried out in triplicate. Data are expressed by Mean ± SEM for n = 10 rats * indicated $P < 0.05$; ** $P < 0.01$ and ** $P < 0.001$ *versus* control group.

(Makris and Kefalas, 2004). This variability may be also due to the varieties of carob tree as recently described by El-Hajaji et al. (2010).

On the other hand, the ABTS free radical scavenging activity of different solvent extract was investigated. Our result clearly indicated that the methanol and water fractions presented an inhibition percentage of (64.26% in the seeds and 40.03% in the pulp, respectively), quite higher than other fractions (0.19 to 23.68%), but lesser than that of Trolox (79.41%). The antioxidant capacity of carob extracts is mainly related to their higher level of phenolic compouds in this fraction. These latter are well known for their ability of scavenging free radicals such as superoxide radical ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$) and others ROS (Rodrigo and Bosco, 2006; Seifried et al., 2007). However, a positive correlation between phenolic compounds and antioxidant capacity is common in the majority of natural extracts (Chon et al., 2009; Hamad et al., 2010).

Based on previous results and knowing that carob pod is formed by 90% of pulp and 10% of seeds, we selected the aqueous fraction, which appears richer in phenolic compounds to investigate the antioxidant effects of carob on healthy rat. Our results showed that subacute (7 days) treatment with AECP is able of alleviating lipid peroxidation as indicated by decreased MDA levels in the brain and heart. Our results fully corroborated recent study which demonstrated that carob polyphenols protected against decreased lipid peroxidation induced by cisplatin administration (Ahmed, 2010). On the other hand, it has recently been shown by Hasouna et al. (2011) that the ethyl acetate fraction (EACs) of carob leaves prevented against CCl_4 induced hepatic oxidative damage and renal failure in rats. We and others well demonstrated the polyphenols richness in carob fruit (Papagiannopoulos et al., 2004) or leaf (Hsouna et al., 2011). These molecules are the primal source of antioxidant ability of carob, by scavenging free radicals as hydroxyl radical ($OH^{\cdot}$) which is the major cause of lipid peroxidation (Kumazawa et al., 2002).

More importantly, we further showed that aqueous extract of carob induced a depletion of hydrogen peroxide in kidney, liver and brain but not in heart tissues. Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be more toxic if converted

to hydroxyl radical in the cell leading to lipid peroxidation (Gulcin et al., 2003) and oxidative DNA damage (Umemura et al., 1990; Khan and Sultana, 2005).

Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolic compounds, which donate electron to H_2O_2, and reducing it to water (Rice-Evans et al., 1997).

In conclusion, our findings clearly demonstrate the richness in phenolic compounds as well as the large antioxidant capacity of carob fruit extracts (pulp and seeds). We also showed that the AECP presented antioxidant properties in healthy rats by decreasing the tissues lipid peroxidation and hydrogen peroxide contents.

ACKNOWLEDGEMENTS

Financial support of the Tunisian Ministry of Higher Education and Scientific Research are gratefully acknowledged. Financial disclosures: none declared.

REFERENCES

Ahmed MM (2010). Biochemical studies on nephroprotective effect of carob (*Ceratonia siliqua* L.) growing in Egypt. Nat. Sci. 8:41–47.

Avallone R, Plessi M, Baraldi M, Monzani A (1997). Determination of chemical composition of carob (*Ceratonia siliqua*): Protein, fat, carbohydrates and tannins. J. Food Compost. Anal. 10:166-172.

Bahorun T, Gressier B, Trotin F, Brunet C, Dine T, Luyckx M, Vasseur J, Cazin M, Cazin JC, Pinkas M (1996). Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. Arzneimittelforschung 46:1086-1089.

Bravo L, Grados N, Saura-Calixto F (1994). Composition and potential uses of mesquite pods (*Prosopis pallida* L.): Comparison with carob pods (*Ceratonia siliqua* L). J. Sci. Food Agric. 65:303–306.

Chon SU, Heo BU, Park YS, Kim DK, Gorinstein S (2009). Total phenolics level, antioxidant activities and cytotoxicity of young sprouts of some traditional Korean salad plants. Plant Foods Hum. Nutr. 64:25-31.

Custodio L, Fernandes E, Escapa AL, Fajardo A, Aligue R, Albericio F, Neng NR, Nogueira JM, Romano A (2011). Antioxidant and cytotoxic activities of carob tree fruit pulps are strongly influenced by gender and cultivar. J. Agric. Food Chem. 59:7005-7012.

Dingeon B, Ferry JP, Roullet A (1975). Automatic assay of blood sugar by Trinder's method]. Ann. Biol. Clin. (Paris) 33:3-13.

Draper HH, Hadley M (1990). Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 186:421-431.

El-Hajaji H, Lachkar N, Alaoui K, Cherrah Y, Farah A, Ennabili A, El-Bali B, Lachkar M (2010). Antioxidant Properties and Total Phenolic Content of Three Varieties of Carob Tree Leaves from Morocco. Rec. Nat. Prod. 4:193-204.

Farrugia G, Balzan R (2012). Oxidative stress and programmed cell death in yeast. Front. Oncol. 2:1-21.

Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI (2003). Screening of antioxidant and Antimicrobial activities of anise (*Pimpinella anisum* L) seed extracts. Food Chem. 83:371-382.

Hamad İ, Erol-Dayi Ö, Pekmez M, Önay-Uçar E, Arda N (2010). Antioxidant and cytotoxic activities of *Aphanes arvensis* extracts. Plant Foods Hum. Nutr. 65:44-49.

Hartree EF (1972). Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal. Biochem. 48:422-427.

Haseeb N, John S, Gauri SM, Yukio K (2006). Extraction of polyphenols from grape seeds and concentration by ultrafiltration. Sep. Purif. Technol. 48:176-181.

Hsouna AB, Saoudi M, Trigui M, Jamoussi K, Boudawara T, Jaoua S, Feki AE (2011). Characterization of bioactive compounds and ameliorative effects of Ceratonia siliqua leaf extract against CCl_4 induced hepatic oxidative damage and renal failure in rats. Food Chem. Toxicol. 49:3183-3191.

Ikeda M, Nakabayashi K, Shinkai M, Hara Y, Kizaki T, Oh-ishi S, Ohno H (2004). Supplementation of Antioxidants Prevents Oxidative Stress during A Deep Saturation Dive. Tohoku J. Exp. Med. 203:353-357.

Jacobo-Velázquez DA, Cisneros-Zevallos L (2009). Correlations of antioxidant activity against phenolic content revisited: A new approach in data analysis for food and medicinal plants. J. Food Sci. 74:R107-113.

Khan N, Sultana S (2005). Chemomodulatory effect of Ficus racemosa extract against chemically induced renal carcinogenesis and oxidative damage response in Wistar rats. Life Sci. 77:1194-210.

Kumazawa S, Taniguchi M, Suzuki Y, Shimura M, Kwon MS, Nakayama T (2002). Antioxidant activity of polyphenols in carob pods. J. Agric. Food Chem. 50:373-377.

Lobo V, Patil A, Phatak A, Chandra N (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn. Rev. 4:118-126.

Makris DP, Kefalas P (2004). Carob Pods (*Ceratonia siliqua* L.) as a Source of Polyphenolic Antioxidants. Food Technol. Biotechnol. 42:105–108.

Marakis S (1996). Carob bean in food and feed: current status and future potentials - a critical appraisal. J. Food Sci. Technol. 33:365-383.

Migdal C, Serres M (2011). Reactive oxygen species and oxidative stress. Med. Sci. (Paris) 27:405-412.

Nabavi SF, Habtemariam S, Jafari M, Sureda A, Nabavi SM (2012). Protective role of gallic Acid on sodium fluoride induced oxidative stress in rat brain. Bull. Environ. Contam. Toxicol. 89:73-77.

Papagiannopoulos M, Wollseifen HR, Mellenthin A, Haber B, Galensa R (2004). Identification and quantification of polyphenols in carob fruits (*Ceratonia siliqua* L.) and derived products by HPLC-UV-ESI/MSn. J. Agric. Food Chem. 52:3784-3791.

Price ML, Van-Scovo S, Buttler LG (1978). A critical evaluation of the Vanillin reaction as an assay for tannin in sorghum grain. J. Agr. Food Chem. 26:1214-1218.

Re R, Pellergrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1998). Antioxidant activity applying and improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26:1231-1237.

Rejeb MN (1995). Le caroubier en Tunisie : situation et perspectives d'amélioration. In Quel avenir pour 1'amélioration des plantes? Ed. AUPELF-UREF. John ibbey Eurotext. Paris pp.79-85.

Rice-Evans CA, Miller NJ, Paganga G (1997). Antioxidant properties of phenolic compounds. Trends Plant Sci. 2:152-159.

Rodrigo R, Bosco C (2006). Oxidative stress and protective effects of polyphenols: comparative studies in human and rodent kidney. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 142:317-327.

Seifried HE, Anderson DE, Fisher EI, Milner JA (2007). A review of the interaction among dietary antioxidants and reactive oxygen species. J. Nutr. Biochem. 18:567-579.

Silanikove N, Gilboa N, Nitsan Z (2001). Effect of polyethylene glycol on rumen volume and retention time of liquid and particulate matter along the digestive tract in goats fed tannin-rich carob leaves (*Ceratonia siliqua*). Small Rumin. Res. 40:95-99.

Turnbull LA, Santamaria L, Martorell T, Rallo J, Hector A (2006). Seed size variability: from carob to carats. Biol. Lett. 2:397-400.

Umemura T, Sai K, Takagi A, Hasegawa R, Kurokawa Y (1990). Oxidative DNA damage, lipid peroxidation and nephrotoxicity induced in the kidney after ferric nitilotriacetate administration. Cancer Lett. 54:95-100.

Zunft HJ, Luder W, Harde A, Haber B, Graubaum HJ, Gruenwald J (2001). Carob pulp preparation for treatment of hypercholesterolemia. Adv. Ther. 18:230–236.

Zunft HJ, Luder W, Harde A, Haber B, Graubaum HJ, Koebnick C, Grunwald J (2003). Carob pulp preparation rich in insoluble fibre lowers total and LDL cholesterol in hypercholesterolemic patients. Eur. J. Nutr. 42:235–242.