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Therapeutic Bio-screening of the Bioactive Ingredients of *Berberis vulgaris*

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ABSTRACT

Barberry (*Berberis vulgaris* L.; family *Berberidaceae*) is a well known plant with traditional herbal medical history. The objectives of the present study were to explore the phytochemical constituents of barberry and to bioscreen its crude extracted bioactive ingredients. The effect of barberry's ingredients on lipid peroxidation in chicken liver and human semen acetylcholinesterase (AChE) and α-gulcosidase activities were spectrophotometrically determined. Our results showed that barberry contains a 71% total phenolic content, including alkaloids (2.6%), flavonoids (4.9%) and saponin (0.3%). Barberry extract showed potent antioxidative capacity through a decrease in thiobarbituric acid reactive species (TBARS) in liver homogenate, human seminal plasma and spermatozoa by 62.5, 72.6 and 96.5%, respectively. Hepatic α-glucosidase activity was enhanced by 51.4% while AChE activity was inhibited by 71%. This work demonstrates the potential of the bioactive ingredients of barberry on suppressing lipid peroxidation, suggesting a promising use in the treatment of hepatic oxidative stress, Alzheimer and idiopathic male factor infertility.

Keywords: acetylcholinesterase, *Berberidaceae*, idiopathic male, oxidative stress
Abbreviations: AChE, Acetylcholine esterase; **BHT**, butylated hydroxytoluene; **BRB-chloride**, berberine chloride; **dH_2O**, distilled water; **TBA**, thiobarbituric acid; **TBARS**, thiobarbituric acid reactive species; **TCA**, trichloroacetic acid; **α-GI**, α-glucosidase inhibitor

INTRODUCTION

Nature has been a source of medicinal agents since the beginning of time. The World Health Organization (WHO) estimates that herbal medicine is still the most common source for primary health care of about 75-80% of the world's population, mainly in the developing countries, because of better cultural acceptability, better compatibility with the human body and fewer side effects (Yadav and Dixit 2008). In ancient oriental medicine, root, leaves, aerial part and even the whole plant have been used to treat various diseases (Mohamed *et al.* 2010). Herbal medicine offers therapeutics for age-related disorders like memory loss, osteoporosis, immune disorders, etc. for which no modern medicine is available (Kamboj 2000). Natural products play an important role in drug development programs of the pharmaceutical industry (Baker *et al.* 1995), where over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros 1982). The amazing structural diversity among their active components make them a useful source of novel therapeutic compounds, thus researchers with interest in natural products have intensified their efforts towards scientific evaluation of traditional medicines (Saeed *et al.* 2007). Furthermore, medical effects are not necessarily restricted to a single plant chemical, since the biological activity and clinical value of the whole plant, as in medicinal herbalism, is also being pursued (Hanachi and Golkho 2009).

Berberis vulgaris L. is considered as one of a well known medicinal plant with traditional herbal medical history and used by many civilizations as a curative herbal remedy in homeopathic systems of medicine (Ivanovska and Philipov 1996). The identification of certain alkaloids and phenolic compounds in barberry somehow provides an alternative method for medicine and remedies. Those therapeutic compounds could lead to the development of new drugs derived from that plant, which is believed to be safer and more effective (Hanachi *et al.* 2008).

Table 1 Compounds isolated from *Berberis vulgaris*.

Compound	Nature	Reference
Aromoline	Alkaloid	Koike *et al.* 1982
Berbamine	Alkaloid	Akasu *et al.* 1976; Wong *et al*. 1992
Berberine	Alkaloid	Wu *et al*. 1977; Yesilada *et al*. 2002; Wang *et al*. 2004; Sudheer *et al*. 2006
Berlambine	Alkaloid	Gasparec *et al*. 1982
Bervulcine	Alkaloid	Werner 1963
Columbamine	Alkaloid	Pavelka and Sme′kal 1976
Hydroxycanthine	Alkaloid	Hagen *et al.* 1989
Isocorydine	Alkaloid	Marsaioli *et al.* 1979
Oxyberberine	Alkaloid	Cushman and Dekow 1979
Oxycanthine	Alkaloid	Hearth *et al.* 1987
Palmatine	Alkaloid	Pavelka and Sme′kal 1976
Quercentin	Flavonoid	Wu *et al.* 1977
Rutin	Flavonoid	Gasparec *et al.* 1982
(-)-tejedine	Alkaloid	Kametani *et al.* 1969
Yatrorizine	Alkaloid	Gasparec *et al.* 1982
Aqueous crude extract		Fatehi *et al.* 2005

Studies carried out on the chemical composition of barberry show that the most important constituents are isoquinoline alkaloids such as berberine, berbamine and palmatine (**Table 1**). Berberine represents one of the most studied among the naturally occurring protoberberine alkaloids since it possesses a wide range of biochemical and pharmacological activities against cardiac disease, stroke, diabetes, hyperlipidemia, chronic inflammation (Zhu and Qian 2006), hypertension, tumors and HIV (Wongbutdee 2008). Barberry also has anti-protozoal, chloretic, cholagogue, cardiotonic, anti-cholinergic, anti-arrhythmic effects and anti-platelet aggregation (**Table 2**) (Wongbutdee 2008).

The nutritional values such as vitamin components and antioxidant compounds in barberry plant might be invalua-

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Table 2 The pharmacological effects of *Berberis vulgaris* (NAPALERT; Natural Products Alert Database).

System	Effect	Part of plant	Preparation	Reference
Cardiovascular	Hypotensive activity	Dried root	Alkaloid fraction	NAPALERT
		Dried fruit	Aqueous extract	Fatehi-Hassanabad *et al*. 2005
Gastrointestinal	Gastric secretory stimulation	Root	Ethanol–H_2O (67%) extract	NAPALERT
Endocrine	Choleretic activity in rat	Dried root	Total alkaloids	NAPALERT
	Choleretic activity	Stem bark		NAPALERT
	Increases tone of the digestive tract and gives rise to increased and irregular peristalsis	Dried root		NAPALERT
	Anticholinergic activity in guinea pig ileum	Dried fruit	Decoction	NAPALERT
	Menstruation induction effect in guinea pig	Stem	Ethanol (95%) extract	NAPALERT
	Uterine stimulant effect in cat, rabbit and guinea-pig	Leaf	Ethanol-acetone (50%) extract	NAPALERT
Immune system	Antibody formation suppression in mouse	Dried root	Alkaloid fraction	APALERT
	Antiinflammatory activity	Root	Alkaloid fraction	Ivanovska and Philipov 1996
Organisms	Complement alternative pathway inhibition	Root	Ethanol (100%) extract	Ivanovska and Philipov 1996
	Delayed type cutaneous hypersensitivity inhibition		Alkaloid fraction and ethanol (95%) extract Alkaloid fraction	NAPALERT
Central nervous system	Antipyretic activity in rat	Dried bark	Alkaloid fraction	NAPALERT
		Dried fruit	Ethanol (95%) extract	NAPALERT
	Narcotic antagonist activity	Dried root		NAPALERT
	Sedative	Fruit		Fatehi-Hassanabad *et al*. 2005
Renal	Diuretic activity in rat	Dried bark	Alkaloid fraction	NAPALERT
Other	Toxicity assessment in mouse – LD_{50}= 520.0 mg/kg	Dried root	Alkaloid fraction	NAPALERT
	Toxicity assessment in mouse – LD_{50}= 2.6 ± 0.22 g/kg b.w.			Peychev 2005
Male reproduction	Idiopathic male factors due to oxidative damage	Root	Crude methanolic (95%) extraction Crude acetic acid (5%) extraction	El-Sayed *et al.* 2010

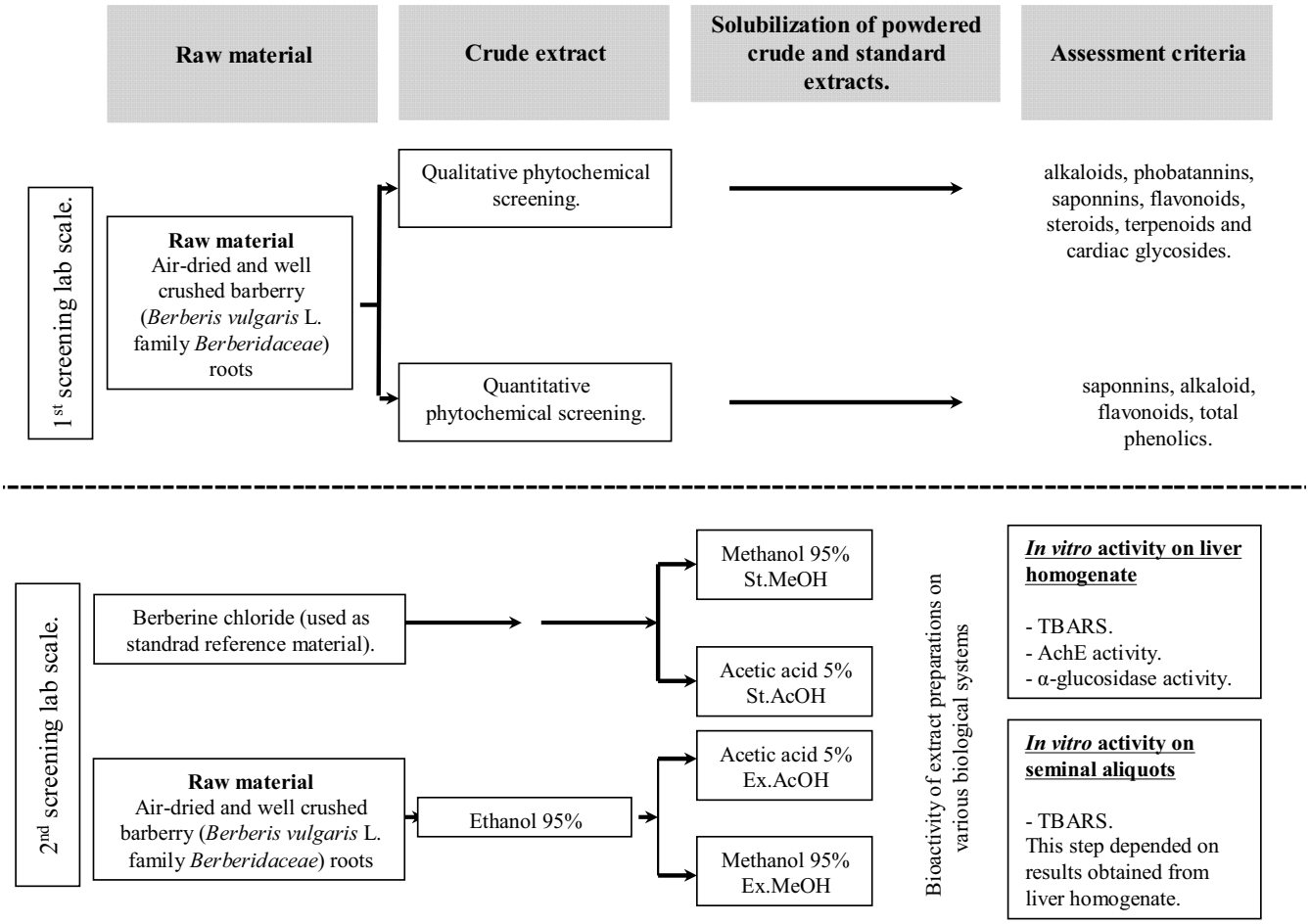

Fig. 1 Experimental design: Phytochemical screening, extraction, solubilization and assessment of barberry bioactive ingredients. St.MeOH, standard dissolved in methanol; St.AcOH, standard dissolved in acetic acid; Ex.MeOH, barberry extract dissolved in methanol; Ex.AcOH, barberry extract dissolved in acetic acid.

ble for treating diseases. Barberry not only exhibited varying degrees of antioxidant properties, but antioxidant capacities also varied according to solvents used for the extraction method (Maznah *et al*. 1999).

The aim of this study was to explore the phytochemical constituents of barberry and to bioscreen its crude extracted bioactive ingredients (**Fig. 1**). An innovative method was designated to investigate the effect of barberry's' extracts on the seminal antioxidant capacity.

MATERIALS AND METHODS

Materials and chemicals

Barberry were collected from the fields and authenticated by Prof. Salma Eldareir, Botany Department, Alexandria University, Egypt. Firstly, the intact plant was removed from the soil, roots were washed more than once and the plant was firmly pressed between paper towels. Then the plant roots were separated, packed within sealable plastic bags and kept out of direct sunlight. Seminal plasma of healthy individuals and spermatozoa samples were collected by Dr. Sherif S Said (Integrated Sterility Centre of Alexandria, Egypt). Liver was obtained from Balady (BB) chicken, one of the local Egyptian chicken strains, and then 1 g was homogenized in 9 mL 0.1 M phosphate buffer, pH 7.4 and stored at -20°C. Trichloroacetic acid (TCA), thiobarbituric acid (TBA), berberine chloride, butylated hydroxytoluen (BHT), acetylthiocholine iodide (ACTI), *p*-nitrophenyl-β-D-glucopyranoside (PNPG) and 5,5′-dithiobis 2-nitrobenzoic acid (DTNB), were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). HAM's Nutrient Mixture F-10 was purchased from Euro-Lone Chemical Co. (UK.). Organic solvents of HPLC-grade like ethanol 95%, methanol and petroleum ether were brought from Merck (USA). All other chemicals and reagents were of analytical grade.

Phytochemical screening of barberry

Plant roots were separated and dried at room temperature, powdered, sieved, and stored prior to further use. Dried barberry roots were phytochemically screened for alkaloids, phobatannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides (Edeoga *et al.* 2005).

Extraction of barberry materials

The dried powdery roots of barberry was exhaustively defatted with petroleum ether and subjected to steam distillation method for ethanolic gradient extraction with Soxhlet apparatus (Upadhyay and Dhawan 1998). The ethanolic extract was concentrated to minimum volume using rotary evaporator (Büchi, Switzerland) then lyophilized (DISHI, DS-FD-SH10, Xi'an Heb Biotechnology Co, China) to obtain a powder extract of barberry (25%). Both barberry powder and berberine chloride (as a standard) were solubilized at 0.1% in two organic solvents with different polarities: methanol (95%) and acetic acid (5%). The barberry extract were kept at -20°C until subjected to further biochemical analysis.

In vitro induction of lipid peroxidation in biological systems

In vitro lipid peroxidation was induced in three biological systems such as chicken liver, healthy human seminal plasma and spermatozoa as described by Tappel and Zalkin (1959) with some modifications as follows:

1. Lipid peroxidation in liver homogenate

2 mL of barberry extract (test), the organic solvent (control) or distilled water (dH_2O) (blank) were incubated with equal volume of liver homogenate for about 45 min at 37°C. *In vitro* tissue lipid peroxidation was induced by adding H_2O_2 and ferrous sulphate ($FeSO_4 \cdot 7H_2O$) at a final concentration of 1 and 0.5 mM, respectively, in both test and control reaction mixtures. After an incubation period of about 30 min at 37°C, BHT at a final concentration of 0.02% was added and mixed carefully to stop the peroxidation reaction. The mixtures were centrifuged at 3000 rpm for 15 min, and then 1 mL of the resultant supernatant was mixed with 1 mL of TCA (15%) followed by centrifugation at 3000 rpm for 10 min.

2. Lipid peroxidation in seminal plasma and spermatozoa

100 µL of each prepared extract (test), organic solvent (control) or saline (blank) were incubated for 15 min with an equal volume of healthy Egyptian human semen samples followed by addition of 400 µl of HAM's Nutrient Mixture F-10. Then the reaction mixtures were centrifuged under cooling at 3000 rpm for 30 min. Supernatants were carefully separated from the spermatozoa's pellets. Then, spermatozoa's pellets were suspended in about 500 µL of sodium phosphate buffer, 0.1 M, pH 7.4. For 500 µL seminal plasma (supernatant) or the suspended spermatozoa's pellet, 1 mL of TCA (15%) was added and the solutions were centrifuged at -4°C for 10 min to obtain protein free supernatant.

Biochemical assays

1. Determination of AChE activity

AChE activity was measured according to the method of Ellman *et al.* (1961). 130 µL phosphate buffer (0.1 M pH 7.4) were added to a mixture of 20 µL of liver homogenate and 20 µL of barberry extract (test) or organic solvent (control), then incubated for 45 min at 37°C. 5 µL of substrate ACTI (75 mM) were added, mixed well and incubated for 15 min at 37°C. 60 µL DTNB (0.32 mM) were added and left for 5 min. The absorbance was measured at 405 nm and the specific activity was calculated.

2. Determination of α-glucosidase activity

Method mentioned by Han and Srinivasan (1969) was carried out with a slight modification to estimate the effect of barberry extract on α-glucosidase (EC 3.2.1.20) activity. 100 µL of barberry extract (test), organic solvents (control) or dH_2O (blank) were diluted with 2.5 mL 0.1 M phosphate buffer pH 7.4. 100 µL of liver homogenate were added, mixed well and incubated in a water bath with the reaction mixture at 30°C for 5 min. 500 µL PNPG, 5 mM, was added and the reaction was allowed to proceed for 15 min. The reaction was stopped by the addition of 2 mL of 1 M Na_2CO_3. The producing color was spectrophotometrically detected at 400 nm. A unit of enzyme activity was defined as nmoles of *p*-nitrophenol released/min.

3. Determination of TBARS level in induced lipid peroxidation

TBARS was determined in liver, protein free supernatant of seminal plasma and spermatozoa samples according to the method described by Tappel and Zalkin (1959). 1 mL of protein free supernatant was mixed with 500 µL TBA (0.7%), heated in boiling water bath for 45 min, cooled and the colour in the supernatant was dead at 532 nm. The TBARS level was calculated against with a control without the extract according to the following equation: TBARS level (nmol/ml) = At / 0.156.

Statistical analysis

Data are expressed as the standard deviation S.D. One-way analysis of variance (ANOVA) followed by Student Newman-Keul's test, which was provided by Primer of Biostatistics program (Version 5). The differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of barberry's roots revealed the presence of alkaloids, flavonoids, saponin, phenolic contents, terpenoids and cardiac glycosides**.** However, steroid and phobatannins were not detected. The percentage of alkaloids, flavonoids, saponin and total phenolic content were 2.55, 4.9, 0.3 and 70.9%, respectively (**Table 3**). All these phytochemical constituents would act in synergy in

Table 3 Quantitative phytochemical screening of barberry*.

Components**	% Concentration ±SD
Saponin	0.3 ± 0.5
Alkaloid	2.6 ± 0.4
Flavonoid	4.9 ± 0.5
Total phenolic content	71 ± 11

* Values are expressed as % of dry raw weight

** Data expressed as ± standard deviation "SD", $P < 0.001$.

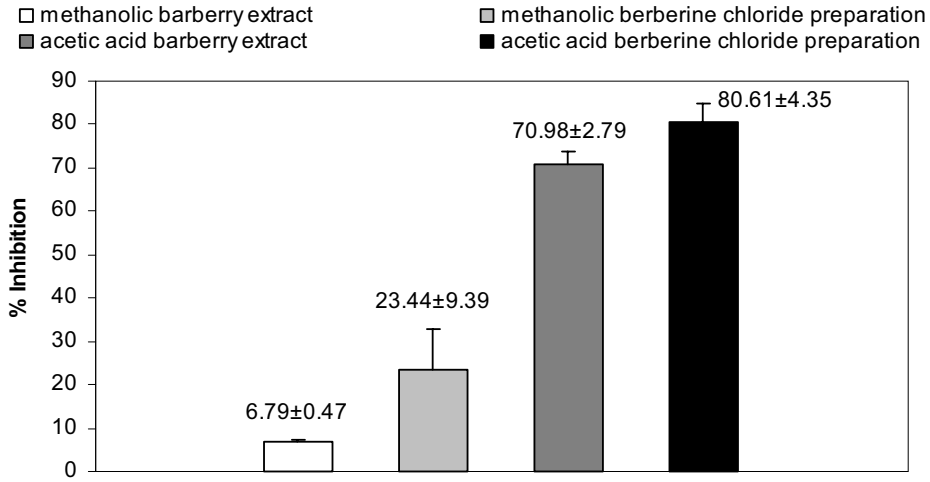

Fig. 2 Effect of the prepared extracts (methanolic and acetic acid) on hepatic AChE. Values represent the mean of three replicates ± standard deviation (SD) ($P < 0.05$).

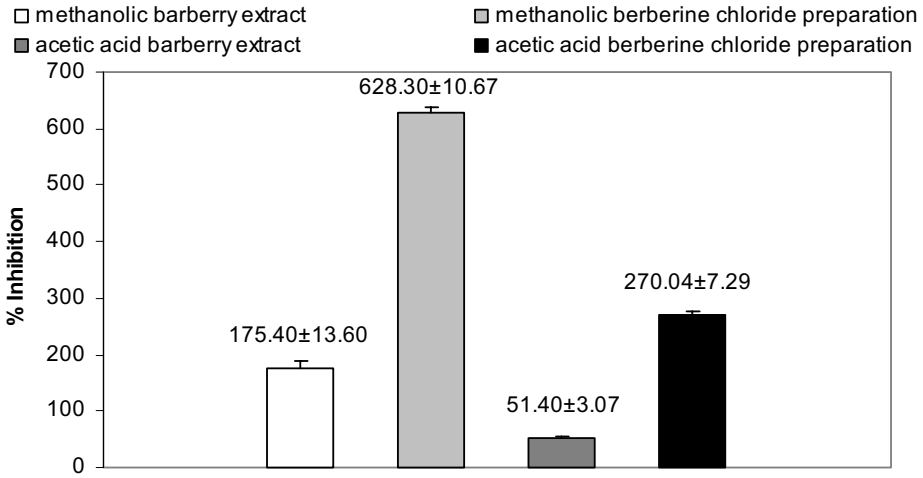

Fig. 3 Effect of the prepared extracts (methanolic and acetic acid) on hepatic α-Glucosidase activity. Values represent the mean of three replicates ± standard deviation (SD), significant difference detected at $P < 0.05$.

order to increase barberry's bioactivity such as antioxidant, antimicrobial, anticholinergic, anti-diabetic, etc. (Wongbutdee 2008). Barberry polysaccharides, cardiac glycosides were clinically used to increase contractile force in patients with cardiac disorders through a mechanism of action involving inhibition of the plasma membrane Na^+/K^+-ATPase leading to alterations in intracellular K^+ and Ca^{2+} levels (McConkey *et al.* 2000; Reuter *et al.* 2001).

The barberry crud extract and berberine chloride solution were prepared at a concentration of (0.1%) in methanol or acetic acid to explore polarity dependent effects.

Anticholinergic activity of the study preparations is shown in **Fig. 2**, where the acetic acid preparations of barberry crude extract and berberine chloride were significantly inhibited the AChE activity by 71 and 80.6%, respectively. These potent anticholinergic properties of barberry are due to its polyphenolic derivatives. Hence barberry might have a therapeutic potential for the treatment of Alzheimer's disease. Ghareeb *et al.* (2010) stated that barberry has a competitive inhibitory ability suggesting its use to alleviate over activity of AChE in dementia patients.

The methanolic and acetic acid preparations of the plant crude extract increased the α-glucosidase activity by 175.4 and 51.4%, respectively, at $P < 0.005$ (**Fig 3**). The great enhancement for α-glucosidase enzyme activity provides an effective way in enzyme replacement therapy of glycogen storage disease type II that caused by lysosomal acid [α]-glucosidase deficiency, where patients have a rapidly fatal or slowly progressive impairment of muscle function (Bijvoet *et al.* 1998).

As shown in **Fig. 4**, acetic and methanolic extracts of barberry were potent inhibitors of lipid peroxidation induced by Fe^{2+} and H_2O_2 in liver homogenate, healthy human seminal plasma and spermatozoa.

Experiments were done to demonstrate the hepatoprotective potential of barberry extracts. Both crude extract and berberine chloride dissolved in acetic acid showed a significant decrease at $P < 0.005$ in liver TBARS by 62.5 and 74.1%, respectively, compared to those dissolved in methanol. This variation in the bioactivity between the two different preparations could be a result of polarity difference between acetic acid and methanol where organic acids' polarity is much greater than that for alcohols. That observation is supporting by the hypothesis of the ability for most polar solvents to dissolve the most polar bioactive ingredient (Sarker and Nahar 2007).

Hanachi and Golkho (2009) reported that the most polar solvent dissolving several compounds of different polarity such as acids, sugars or glycosides which may be contributed to the total phenolic content of the extract and represented the highly antioxidant properties. Depending on this we speculated that acetic acid extract preparations exhibited appreciable antioxidantive activity against the generation of cellular oxidized lipid particles. Therefore, acetic acid extract was selected for further experiments carried out on human seminal plasma and spermatozoa.

We noticed that acetic acid barberry extract was significantly decreased the TBARS level in seminal plasma and spermatozoa by 72.58 and 96.46%, respectively. This significant antioxidant activity of barberry could be attributed to its high contents of active phytochemicals as previously mentioned. Furthermore, our data reflecting the possibility

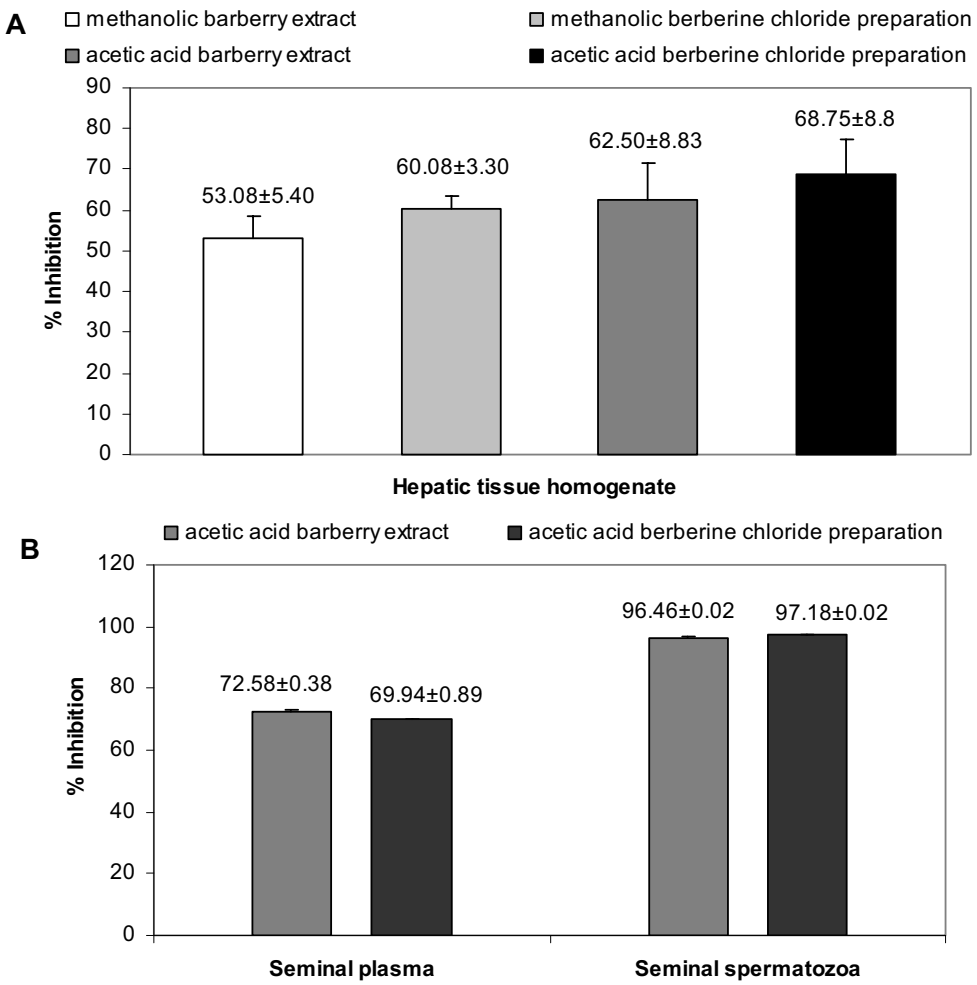

Fig. 4 Total antioxidant capacity of the prepared extracts (methanolic and acetic acid) on hepatic homogenate (A), human seminal plasma and spermatozoa (B). Values represent the mean of three replicates ±standard deviation (SD), significant difference detected at $P < 0.05$.

use of barberry extract as a natural antioxidant agent for treatment of idiopathic male factor infertility as well as in assisted reproductive technology (**Table 3**).

CONCLUDING REMARKS

The data obtained support the hypothesis that the bioactive ingredients of barberry have beneficial effects on suppressing hepatic and seminal lipid peroxidation. Barberry displaying such capacities may be used in treatment of hepatic, cardiovascular, hyperglycemic and Alzheimer diseases as well as idiopathic male factor infertility. Further studies will be carried out to identify and characterize the potential bioactive phytochemicals of barberry.

REFERENCES

Ahmed M, Alamgeer, Sharif T (2009) A potential adjunct to insulin: *Berberis Lycium* role. *Diabetologia Croatica* **38**, 13-17

Akasu M, Itokawa H, Mitiiti F (1976) Biscocalaurine alkaloids in callus tissues of *Stephania cepharantha*. *Phytochemistry* 15, 471-473

Bijvoet A, Kroos M, Pieper F, Vliet M, Boer H, Ploeg A, Verbeet M, Reuser A (1998) Recombinant human acid α-glucosidase: high level production in mouse milk, biochemical characteristics, correction of enzyme deficiency in GSDII KO mice. *Human Molecular Genetics* **7**, 1815-1824

Gasparec K, Zvonimir G (1982) The ultraviolet and visible absorption spectra of berberrubine. *Canadian Journal of Chemistry* **60**, 970-975

Cocuzza M, Sikka S, Athayde K, Agarwal A (2007) Clinical relevance of oxidative stress and sperm chromatin damage in male infertility: an evidence based analysis. *Brazilian Journal of Urology* **33**, 603-621

Edeoga H, Okwu D, Mbaebie B (2005) Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* **4**, 685-688

Fatehi M, Saleh T, Fatehi-Hassanabad Z, Farrokhfal K, Jafarzadeh M, Davodi S (2005) A pharmacological study on *Berberis vulgaris* fruit extract. *Journal of Ethnopharmacology* **102**, 46-52

Fatehi-Hassanabad Z, Jafarzadeh M, Tarhini A, Fatehi M (2005) The antihypertensive and vasodilator effects of aqueous extract from *Berberis vulgaris* fruit on hypertensive rats. *Phytotherapeutic Research* **19**, 222-225

Ghareeb D, Newairy A, El-Rashidy F, Hussein H, Ali A (2010) Efficacy of natural extracts of *Ginkgo biloba* and barberry and a synthetic derivative of genistein (ipriflavone), as acetylcholinesterase inhibitors, comparative study with Aricept® effect. *Journal of Biochemistry and Biotechnology* **1**, 15-20

Guffanti A, Corpe A (1976) Partial purification and characterization of A-Glucosidase from *Pseudomonas fluorescens*. *World Archive Microbiological* **107**, 269-276

Han W, Srinivasan R (1969) Purification and characterization of beta-glucosidase of Alcaligenesfaecalis. *Journal of Bacteriology* **100**, 1355-1363

Hanachi P, Golkho SH (2009) Using HPLC to determination the composition and antioxidant activity of *Berberis vulgaris*. *European Journal of Scientific Research* **29**, 47-54

Hanachi P, Fauziah O, Motalleb G (2008) Evaluation of sodium, potassium and apoptotic cells in the liver of hepatocarcinogenic rats treated with *Berberis vulgaris*. *Iranian Journal of Basic Medical Science* **11**, 49-54

Ho W, Ky H, Yeap S, Rahim R, Omar A, Ho C, Alitheen N (2009) Traditional practice, bioactivities and commercialization potential of *Elephantopus scaber* Linn. *Journal of Medicinal Plants Research* **3**, 1212-1221

Ivanovska N, Philipov S (1996) Study on the antiinflammatory action of *Berberis vulgaris* root extract, alkaloid fractions, and pure alkaloids. *International Journal of Immunopharmacology* **18**, 552-561

Kamboj V (2000) Herbal medicine. *Current Science* **78**, 35-39

Kametani T, Iida H, Sakurai K, Kano S, Ihara M (1969) The nuclear magnetic resonance spectra and optical rotatory dispersion of berbamunine, magnoline and two diastereoisomers. *Chemical and Pharmaceutical Bulletin* **17**, 2120-2125

Koike L, Marsaioli J, Ries Francisco de M (1982) Carbon-13 NMR spectroscopy and conformational analysis of the daphnoline-repandine class of bis(benzylisoquinoline) alkaloids. *Journal of Organic Chemistry* **47**, 4351-4353

Mankil J, Moonsoo P (2007) Acetylcholinesterase inhibition by flavonoids from *Agrimonia pilosa*. *Molecules* **12**, 2130-2139

Maznah I, Teoh L, Loh P (1999) Determination of total antioxidant activity of selected local medicinal plants. In: *Proceeding Herbs an International Con-*

ference and Exhibitions **9**, 124-128

McConkey D, Lin Y, Nutt L, Ozel H, Newman R (2000) Cardiac glycosides stimulate Ca^{2+} increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. *Cancer Research* **60**, 3807-3812

Mohamed A, Khalill A, El-Beltagy H (2010) Antioxidant and antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*). *Grasas y Aceites* **61**, 67-75

Mohsen I, Hossein H (2007) Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. *Phytotherapy Research* **22**, 999-1012

Pavelka S, Sme′kal E (1976) The fluorescence properties of protoberberine and tetrahydrotoberberine alkaloid. *Collection of Czechoslovak Chemical Communications* **41**, 3157-3169

Peychev L (2005) Pharmacological investigation on the cardio-vascular effects of *Berberis vulgaris* on tested animals. *Pharmacia* **52**, 118-121

Reuter H, Henderson S, Han T, Ross R, Goldhaber J, Philipson K (2002) The Na^{+}-Ca^{2+} exchanger is essential for the action of cardiac glycosides. *Journal of the American Heart Association* **90**, 305-308

Saeed A, Najma S, Saima S (2007) The berberis story: *Berberis vulgaris* in therapeutics. *Pakistan Journal of Pharmaceutical Sciences* **20**, 83-92

Sarker D, Nahar L (2007) *Chemistry for Pharmacy Students. General, Organic and Natural Product Chemistry*, John Wiley and Sons Ltd., England, 20 pp

Sudheer K, Mantena S, Sharma D, Santosh K (2006) Berberine, a natural product, induces Gi-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells. *Molecular Cancer Therapy* **5,** 296-308

Tappel L, Zalkin H (1959) Inhibition of lipid peroxidation in mitochondria by vitamin E. *Archives of Biochemistry and Biophysics* **80**, 333-336

Wang F, Zhao G, Cheng L, Zhou HY, Fu LY, Yao WX (2004) Effects of berberine on potassium currents in acutely isolated CAI pyramidal neurons of rat hippocampus. *Brain Research* **999**, 91-97

Werner D (1963) New alkaloids from berberis vulgaris. *Naturwissenschaften* **50**, 585-595

Wong C, Seow W, O'Callaghan J, Thong Y (1992) Comparative effect of tetrandrine and berbamine on subcutaneous air pouch inflammation induced by interleukin-1, tumor necrosis factor and platelet-activating factor. *Agent Actions* **36**, 112-118

Wongbutdee J (2008) Physiological effects of berberine. *Thailand Pharmaceutical Health Science Journal* **4**, 78-83

Wu N, Beal L, Leu L, Doskotch W (1977) Alkaloids of *Thalictrum*. XXI. Isolation and characterization of alkaloids from the roots of *Thalictrum podocarpum*. *Lloydia* **40**, 508-514

Yadav P, Dixit K (2008) Recent approaches in herbal drug standardization. *International Journal of Integrative Biology* **2**, 195-203

Yesilada E, Kupeli E (2002) *Berberis crataegina* DC root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats. *Journal of Ethnopharamacology* **79**, 237-248

Yoshikawa Y, Hirata R, Yasui H, Sakurai H (2009) A-glucosidase inhibitory effect of anti-diabetic metal ions and their complexes. *Biochemie* **91**, 1339-1341

Zhu F, Qian C (2006) Berberine chloride can ameliorate the spatial memory impairment and increase the expression of interleukin-1 beta and inducible nitric oxide synthase in the rat model of Alzheimer's disease. *BMC Neuroscience* **7**, 1-9