Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: Kundan Singh Bora & Anupam Sharma (2011) Evaluation of antioxidant and free-radical scavenging potential of *Artemisia absinthium*, Pharmaceutical Biology, 49:12, 1216-1223, DOI: 10.3109/13880209.2011.578142

To link to this article: https://doi.org/10.3109/13880209.2011.578142

Pharmaceutical Biology, 2011; 49(12): 1216–1223

ISSN 1388-0209 print/ISSN 1744-5116 online
DOI: 10.3109/13880209.2011.578142

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RESEARCH ARTICLE

Evaluation of antioxidant and free-radical scavenging potential of *Artemisia absinthium*

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Abstract

Context: Currently there has been an increased global interest to identify antioxidant compounds for use in preventive medicine and the food-industry that are pharmacologically potent and have low or no side effects. As plants produce significant amounts of antioxidants to prevent oxidative stress, they represent a potential source of new compounds with antioxidant activity.

Objective: The current study was designed to evaluate the methanol extract of *Artemisia absinthium* Linn. (Asteraceae; MAB) for its *in vitro* free-radical scavenging effects using different classical assays, and *in vivo* antioxidant activity, using global cerebral ischemia and reperfusion (I/R)-induced oxidative stress in mice.

Materials and methods: The *in vitro* scavenging activity was studied on superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide radical, and reducing power. Further, in the *in vivo* studies, the animal model of global cerebral I/R was established by occluding the bilateral common carotid artery for 15 min followed by 24-h reperfusion. The thiobarbituric acid reactive substances (TBARS) concentration, superoxide dismutase (SOD) activity and glutathione (GSH) content were determined by colorimetric assays.

Results: In the *in vitro* assays the, MAB showed significant ($p < 0.05$) superoxide anion, hydrogen peroxide, hydroxyl and nitric oxide radical scavenging activities, and significant reducing power. Furthermore, in the *in vivo* studies, oral administration of MAB (100 or 200 mg/kg) inhibited cerebral I/R-induced oxidative stress by decreasing TBARS, and restoring levels of SOD and GSH.

Conclusion: The results indicated that *A. absinthium* possess potent antioxidant properties, and may be used as a protective agent against disorders associated with oxidative stress.

Keywords: Cerebral ischemia/reperfusion, flavonoids, oxidative stress, phenolic compounds, reactive oxygen species, superoxide dismutase, thiobarbituric acid reactive substances

Introduction

Reactive oxygen species (ROS) readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins, and DNA. This oxidative damage is a crucial etiological factor implicated in several chronic human diseases, namely cardiovascular diseases, rheumatism, diabetes mellitus, cerebrovascular diseases, and cancer (Sabir & Rocha, 2008; Yang et al., 2010). Based on a growing interest in free-radical biology and the lack of effective therapies for most chronic diseases, a study of the usefulness of antioxidants in protection against these diseases is warranted. Antioxidants are chemical substances that reduce or prevent oxidation. They have the ability to counteract the damaging effects of free-radicals in tissues and thus are believed to protect against cancer, cerebrovascular diseases, arteriosclerosis, heart disease, and several other diseases (Bandyopadhyay et al., 2007).

Many antioxidant compounds, naturally occurring in plant sources have been identified as free-radical scavengers (Verma et al., 2010). A number of synthetic antioxidants, such as 2- and 3-*tert*-butyl-4-methoxyphenol (butylated hydroxyanisole; BHA) and *tert*-butylhydroquinone have been added to foodstuffs, but because of

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(Received 29 December 2010; revised 16 March 2011; accepted 01 April 2011)

toxicity issues, their use is being questioned. Therefore, attention has been directed towards the development and isolation of natural antioxidants from plant sources. Crude extracts of spices, herbs, and other plant materials rich in polyphenolics are increasingly of interest to the pharmaceutical and food industry because they have the capacity to retard oxidative degradation and thereby improve the quality of pharmaceutical products, and nutritional value of food (Amarowicz et al., 2004).

Many studies reported that phenolic compounds display antioxidant activity as a result of their capacity to scavenge free-radicals (Seyoum et al., 2006; Yang et al., 2010). Phenolic compounds can also act as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system. Therefore, the search for natural antioxidants of plant origin has gained momentum in recent years.

Artemisia absinthium Linn. (Asteraceae), commonly known as wormwood, is an aromatic, perennial undershrub growing naturally in Europe, North America, and Asia. Traditionally, wormwood has been used as an antiseptic, antispasmodic, anticancer, febrifuge, cardiac stimulant, for the restoration of declining mental function and inflammation of the liver, and to improve memory (Koul, 1997; Wake et al., 2000).

Phytochemically, *A. absinthium* has been reported to possess essential oil, absinthin, anabsin, anabsinthin, artabsin, and matricin; resins, lactones, and organic acids (Omer et al., 2007). Wormwood also contains flavonoids such as quercetin, rutin (Rice-Evans et al., 1996; Lee et al., 2004), and other flavonoid glycosides (isoquercitrin, quercitin-3-*O*-β-D-glucoside, quercitin-3-*O*-rhamnoglucoside, isorhamnetin-3-*O*-rhamnoglucoside, isorhamnetin-3-glucoside), and phenolic acids such as chlorogenic, syringic, coumaric, salicylic, and vanillic acids (Kordali et al., 2005) are probably involved in the mechanism of free-radical scavenging activity. These pharmacophores have been shown to possess potent antioxidant and free-radical scavenging activity, and anti-inflammatory activity (Kordali et al., 2005).

Pharmacological reports revealed that *A. absinthium* enhance the cognitive ability as evidenced by its nicotinic and muscarinic receptor activity in homogenates of human cerebral cortical membranes (Wake et al., 2000). Hexane-, chloroform-, and water-soluble extracts of the plant exhibited antipyretic activity against subcutaneous yeast injections in rabbits. Moreover, it has been reported that methanol extract of this plant enhanced neurite outgrowth induced by nerve growth factor and PC12D cells (Li & Ohizumi, 2004). Recently, the authors have shown that *A. absinthium* exhibited significant neuroprotective effects against ischemia and reperfusion insult in rats (Kundan & Anupam, 2010).

The aim of the this study was to evaluate the *in vitro* and *in vivo* antioxidant and free-radical scavenging activity, total phenolic and flavonoid content of methanol extract of *A. absinthium*, by using a number of classical assays, and to assess whether *A. absinthium* could be a source of natural antioxidant for pharmaceutical applications.

Materials and methods

Plant materials

Aerial parts of the plant *A. absinthium* were procured from Himalaya Herbs Stores, Saharanpur, Uttar Pradesh, India. Identity of the plant material was authenticated by Dr. B. Singh, scientist at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen of *A. absinthium* has been deposited in the Herbarium-cum-Museum of the University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India. The dried aerial parts of the plant were milled to a fine powder using an electric blender. The methanol extract was prepared by extracting 100 g of powdered plant material using Soxhlet apparatus (20 h). Thereafter, the resulting methanol extract was reduced *in vacuo* (40°C), freeze-dried and stored at 4°C until used. The extract obtained was called MAB.

Chemicals

Nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), BHA, butylated hydroxy toluene (BHT), l- ascorbic acid, nicotinamide adenine dinucleotide (NADH), gallic acid (GA), α-tocopherol, 1,1,3,3-tetraethoxypropane, trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were obtained from Sigma–Aldrich, Chemie Gmbh Munich, Germany. Chloral hydrate was obtained from Reidel–deHaen, Germany. All other chemicals used were analytical grade and were obtained from Merck, Merck, KGaA, Darmstadt, Germany.

Standardization of extract

Determination of total phenolic content

The total phenolic content of MAB was analyzed according to the Folin–Ciocalteu method as described by Cliffe et al. (1994). In brief, MAB was well mixed with 2.5 mL of distilled water, and then 0.5 mL of the Folin–Ciocalteu stock reagent and 1.0 mL of Na_2CO_3 reagent (75 g/L) were added to the mixture. They were then incubated at room temperature for 30 min. The mixture absorbance was spectrophotometrically (Beckman DU 640B, Nyon, Switzerland) measured at 765 nm.

Determination of total flavonoid content

The total flavonoid content of MAB was determined according to colorimetric method as described by Zou et al. (2004). In brief, 0.5 mL of sample solution was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5% $NaNO_2$ solution. After 6 min of incubation, 0.15 mL of 10% $AlCl_3$ solution was added and then allowed to stand for 6 min, followed by adding 2 mL of 4% sodium hydroxide solution to the mixture. Immediately

after water was added to the sample to bring the final volume to 5 mL, the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture absorbance was determined at wavelength 510 nm.

In vitro assays

Superoxide anion scavenging activity

Superoxide anion scavenging activity of the extract was validated according to the method described by Liu et al. (1997). Superoxide radical is generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of NBT. In this experiment, the superoxide radical was generated in 3 mL of Tris–HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 μM), 1 mL NADH (78 μM) and sample solutions of extract (25–75 μg/mL) in water were mixed. The reaction was started adding 1 mL of 10 M PMS to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance was recorded at 560 nm against blank samples. GA was used as a control. The percentage inhibition of superoxide anion radical generation was calculated using the following formula:

$$\text{Inhibition (\%)} = \left(\frac{\text{A0} - \text{A1)}}{\text{A0}} \right) \times 100,$$

where A0 was the absorbance of the control, and A1 was the absorbance in the presence of the sample of MAB and standards.

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Rajeshwar et al. (2005). The assay was performed by adding 0.1 mL ethylenediaminetetraacetic acid, 0.01 mL of $FeCl_3$, 0.1 mL hydrogen peroxide, 0.36 mL of deoxyribose, 1.0 mL of test solutions (10–75 μg/mL) dissolved in distilled water, 0.33 mL of phosphate buffer (50 mM, pH 7.4), and 0.1 mL of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 h. A 1.0 mL portion of the incubated mixture was mixed with 1.0 mL of 10% TCA and 1.0 mL of 0.5% TBA to develop the pink chromogen, which was measured at 532 nm. BHT was used as standard.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured by a spectrophotometric method (Madan et al., 2005). Sodium nitroprusside (5 mmol) in phosphate-buffered saline was mixed with a control without the test compound, but with an equivalent amount of methanol. Test solution of increasing concentrations (10–75 μg/ mL) were dissolved in methanol and incubated at 25°C for 30 min. After 30 min, 1.5 mL of the incubated solution was diluted with 1.5 mL of Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of the nitrite with sulfanilamide and the subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm.

Hydrogen peroxide scavenging activity

The ability of the MAB to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (4 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically at 230 nm. Increasing concentrations of MAB (10–75 μg/ mL) in distilled water was added to hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of MAB and standard compounds:

$$\text{\% Scavenged (hydrogen peroxide)} = \left(\frac{\text{A0} - \text{A1/}}{\text{A0}} \right) \times 100,$$

where A0 was the absorbance of the control, and A1 was the absorbance in the presence of the sample of MAB and standards.

Reducing power assay

The reducing power of MAB was determined according to method of Oyaizu (1986). Briefly, increasing concentrations of the extract and the standard compound (BHT) in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of a potassium ferric cyanide solution (1%, w/v). The mixture was incubated in a water bath at 50°C for 20 min. Then, 2.5 mL of a TCA solution (10%, w/v) was added, and the mixture was then centrifuged at 3000*g* for 10 min. A 2.5 mL aliquot of the upper layer was combined with 2.5 mL of distilled water and 0.5 mL of a ferric chloride solution (0.1%, w/v), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture correlates with greater reducing power.

In vivo biochemical assays

Animals and experimental protocols

Male Swiss albino mice (20–30 g) were maintained on standard environmental conditions, and fed with standard rodent diet and tap water *ad libitum*. They were housed in the institutional animal house and maintained on a 12-h light/dark cycle regulated at 22 ± 2°C room temperature. All studies were performed in accordance with the guidelines on regulation of scientific experiments on animals, as adopted and promulgated by the Animals Ethics Committee of L.R. Institute of Pharmacy.

A total of four groups of seven mice each were employed in the present study. The first group was sham operated (mice were subjected to surgical procedure, a thread

was passed below both carotid arteries but the arteries were not occluded after 15 min, thread was removed and the animal was sutured back and allowed to recover for 24 h). A second group served as control {mice were orally administered vehicle [simple syrup i.p. + Tween 80 (5%), 10 mL/kg] 1 h before subjecting to 15-min global cerebral ischemia by bilateral carotid artery occlusion (BCAO) followed by reperfusion for 24 h}. Third and fourth groups of mice received 100 mg kg^{-1} and 200 mg kg^{-1} doses of MAB, respectively. The vehicle and extracts were administered orally, 60 min before subjecting them to global cerebral ischemia.

Bilateral common carotid arteries occlusion

Animals were subjected to BCAO under chloral hydrate anesthesia (400 mg/kg, i.p.). A cotton thread was passed below each carotid artery. Pulling the ends of thread with constant weight induced global cerebral ischemia. After 15 min of global cerebral ischemia, weight on the thread was removed to allow the reflow of blood through carotid arteries. The incision was sutured back in layers (Himory et al., 1990). Temperature was maintained at 37°C throughout the surgical procedure.

Estimation of thiobarbuturic acid reactive substance (TBARS)

After 24 h-reperfusion, animals were sacrificed and brains were removed, weighted, minced and suspended in a buffer containing 30 mM Tris–HCl and 2.5 mM $CaCl_2$ (pH 7.6). The above mixture was homogenized, and the homogenate was centrifuged at 750*g* to separate cellular debris. The supernatant was accurately divided into two parts. Both portions were centrifuged at 8200*g* to obtain mitochondrial fraction. One was utilized for determination of thiobarbituric acid reactive substances (TBARS) (Yagi, 1982) and the other one was employed for protein estimation (Lowry et al., 1951).

Superoxide dismutase

Superoxide dismutase (SOD) activity was measured by the method of Kakkar et al. (1984). The mouse brains were homogenized in ice-cold sodium pyrophosphate buffer (pH 8.3) in a ratio of 50 mg/mL; 200 μL of this homogenate was used for the assay. The inhibition by SOD of reduction of NBT to blue-colored chromogen in the presence of PMS and NADH was measured at 560 nm. One unit of enzyme activity was defined as the enzyme concentration required inhibiting the absorbance at 560 nm of chromogen production by 50% in 1 min under assay condition, and expressed as specific activity in unit of SOD/min/mg protein.

Reduced glutathione

Reduced glutathione (GSH) in the brain was determined by the method of Jollow et al. (1974). 1.0 mL of PMS (10% w/v) was precipitated with 1.0 mL of sulfosalicylic acid (4%). The samples were kept at 4°C for at least 1 h and, then, subjected to centrifugation at 1200*g* for 15 min at 4°C. The assay mixture contained 0.1 mL of PMS (10%, w/v), 2.7 mL of phosphate buffer (0.1 M, pH 7.4) and 0.2 mL DTNB (40 mg/10 mL phosphate buffer, 0.1 M, pH 7.4) in a total volume of 3.0 mL. The yellow color developed was read immediately at 412 nm. The enzyme activity was calculated as nM DTNB oxidized/min/mg of protein.

Statistical analysis

The results are presented as means ± SD. All parameters of *in vitro* assays were analyzed using Student's *t*-test. Whereas, the data of *in vivo* biochemical assays were statistically analyzed using one-way analysis of variance followed by *post hoc* Tukey's multiple range test; $p < 0.05$ was considered significant.

Results

Extract yield, total phenolic, and flavonoid content

The methanol extract yield was 12.26% (w/w). The total phenolic content was estimated to be 123 ± 0.82 mg GA equivalents per gram of extract from triplicate measurements. The total flavonoid content was expressed in milligrams of rutin equivalents per gram of extract. The total flavonoids content was 24 ± 0.96 mg rutin equivalents per gram of extract from triplicate measurements.

In vitro observations of MAB

Scavenging of superoxide anion radical

In PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Table 1 shows the percentage inhibition of superoxide radical generation at different concentrations (25–100 μg/mL) of MAB compared with the same concentration of GA (standard). MAB exhibited concentration-dependent scavenging activities against superoxide anion radicals generated in PMS-NADH systems. MAB showed significant ($p < 0.05$) superoxide radical scavenging activity (78.1%), at 100 μg/mL concentration.

Scavenging of hydrogen peroxide

Hydrogen peroxide scavenging activity of MAB is shown in Table 2 and compared with that of α-tocopherol as standard. The results indicate that MAB is capable of scavenging hydrogen peroxide in a concentration-dependent manner. From the different concentrations of MAB and α-tocopherol tested for scavenging activity, the EC_{50} value was found to be 106.41 and 18.52 μg/mL, respectively.

Scavenging of hydroxyl radical

MAB exhibited significant ($p < 0.05$) hydroxyl radical scavenging activity in a concentration-dependent manner in the range of 25–100 μg/mL in the reaction mixture with 79.3% scavenging at a concentration of 100 μg/mL (Figure 1).

Scavenging of nitric oxide

Nitric oxide was generated from sodium nitroprusside and measured by Greiss reaction. MAB showed nitric oxide scavenging activity between 25 and 200 μg/mL in a concentration-manner (EC_{50} = 180.71 μg/mL; Table 2). The extract showed a moderate nitric oxide scavenging activity. The percentage of inhibition was increased with increasing concentration of the extract. BHA (EC_{50} = 26.51 μg/mL) was used as a positive control for comparison.

Reducing power

Figure 2 shows the reductive capability of MAB compared to BHT. The reducing power of MAB increased with increasing concentration. At the concentrations of 50, 100, 200 μg/mL of MAB showed lower activities than BHT, and these differences were statistically significant ($p < 0.05$). But at 400 μg/mL concentration of MAB a significant ($p < 0.05$) reducing power was shown.

In vivo observations of MAB

TBARS concentration in brain mitochondrial and supernatant fractions was significantly ($p < 0.05$) elevated in mouse ischemic brain due to ischemia-reperfusion (I/R) insult (Figure 3). Pre-treatment with MAB significantly ($p < 0.05$) decreased the elevated TBARS concentration in brain mitochondrial and supernatant fractions as compared to control group.

SOD and GSH showed a significant ($p < 0.01$) decrease in the control group versus the respective sham group. Pre-treatment with MAB markedly reversed the alterations in biochemical parameters brought about by I/R. The values were almost restored to normal levels with no significant differences versus the sham group. SOD and GSH were significantly ($p < 0.01$) elevated in the MAB (200 mg/kg) treated animals subjected to BCAO and reperfusion injury as compared to control group (Table 3).

Table 1. Superoxide anion radical scavenging activity of MAB and GA by the PMS-NADH-NBT method.

Sample	Concentration (μg/mL)	% Inhibition
MAB	25	19.40 ± 1.02
	50	43.01 ± 1.09
	75	69.50 ± 1.03*
	100	78.12 ± 0.98*
GA	50	65.05 ± 1.01
	75	82.12 ± 1.08
	100	91.60 ± 1.33

Values are presented as mean ± SD.
*$p < 0.05$ compared to standard drug. Statistical analysis was done by using Student's *t*-test.
GA, gallic acid; MAB, methanol extract of *Artemisia absinthium.*

Discussion

Oxidative stress, in which ROS like superoxide, hydrogen peroxide, hydroxyl radical, singlet oxygen, and nitrogen species are generated, is one of the earliest responses to stress. Superoxide anions are the most common free-radicals, generated in a variety of biological systems and the concentration of superoxide anions increases under conditions of oxidative stress (Lee et al., 2002). These anions are produced endogenously by flavoenzymes-like xanthine oxidase, which converts hypoxanthine to xanthine and subsequently to uric acid in I/R (Blaszczyk et al., 1994). The significant decrease in the concentration of the superoxide anion radicals was observed in this study due to the scavenging ability of MAB.

Hydrogen peroxide can be formed *in vivo* by many oxidized enzymes such as SOD. It can cross membranes and may slowly oxidize a number of compounds. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells (Ilhami et al., 2004). Levels of hydrogen peroxide at or below about 20–50 mg seem to have limited cytotoxicity to many cell types. Thus, removing hydrogen peroxide as well as superoxide anion is very important for protection. The EC_{50} value for scavenging activity was 106.41 μg/mL.

Hydroxyl radicals are extremely ROS, capable of modifying almost every molecule in the living cells. They have the capacity to cause strand damages in DNA leading to carcinogenesis, mutagenesis, and cytotoxicity. Moreover, hydroxyl radicals are capable of the quick initiation of lipid peroxidation process as by abstracting hydrogen atoms from unsaturated fatty acids (Aruoma, 1998). In the present observation, MAB exhibited significant ($p < 0.05$) hydroxyl radical scavenging activity in a dose dependent manner.

The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free-radical chain by donating a hydrogen atom (Pin-Der-Duh, 1998). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.

Table 2. Nitric oxide (NO) scavenging and hydrogen peroxide (H_2O_2) scavenging effects of MAB, BHA and α-tocopherol.

Sample	Concentration (μg/mL)	% Inhibition (NO)	% Inhibition (H_2O_2)	$EC50^{NO}$ (μg/mL)	$EC50^{H_2O_2}$ (μg/mL)
MAB	25	14.40 ± 1.08	15.07 ± 1.99	180.71	106.41
	50	21.21 ± 1.05	26.04 ± 1.06		
	75	—	40.31 ± 1.00		
	100	35.32 ± 0.98	47.98 ± 0.91		
	200	56.22 ± 1.02	—		
BHA				26.51	—
α-Tocopherol				—	18.52

BHA, butylated hydroxyanisole; MAB, methanol extract of *Artemisia absinthium.*

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Our data on the reducing power of MAB suggest that it is likely to contribute significantly towards the observed antioxidant effect. Like the antioxidant activity, the reducing power of MAB increased with increasing amount of sample. No significant difference was observed between MAB and BHT.

In addition to ROS, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. Nitric oxide was generated from sodium nitroprusside and measured by Greiss reaction. Scavengers of nitric oxide compete with the oxygen, leading to reduced production of nitric oxide (Govindarajan et al., 2003). The extract showed a moderate nitric oxide scavenging activity. The percentage of inhibition was increased with increasing concentration of the extract.

TBARS, SOD, and GSH were estimated as an index to assess the severity of oxidative damage in the brain tissue, and also the effect of *A. absinthium* on the reversal of the damage produced by BCAO. All these parameters were markedly reversed and restored to near normal levels in the groups pre-treated with MAB. Free-radicals are well investigated in the development of I/R-induced

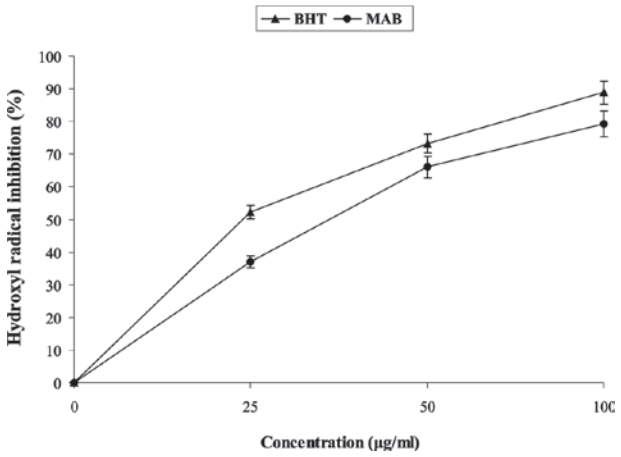

Figure 1. Hydroxyl radical scavenging activity of MAB and BHT (25–100 µg/mL). BHT, butylated hydroxytoluene; MAB, methanol extract of *Artemisia absinthium*.

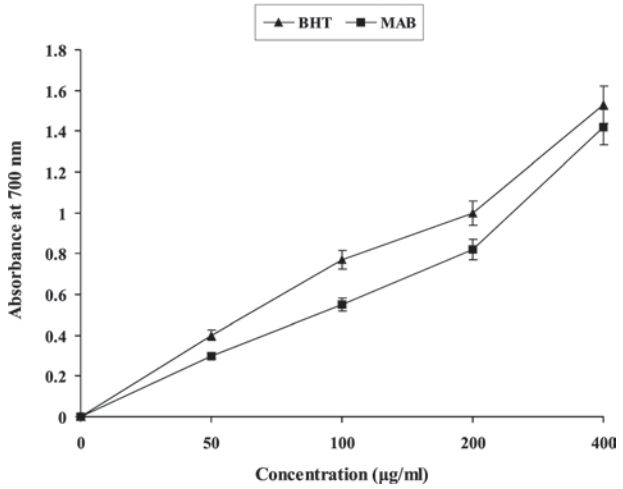

Figure 2. Reducing power of MAB and BHT (50–400 µg/mL). BHT, butylated hydroxytoluene; MAB, methanol extract of *Artemisia absinthium*.

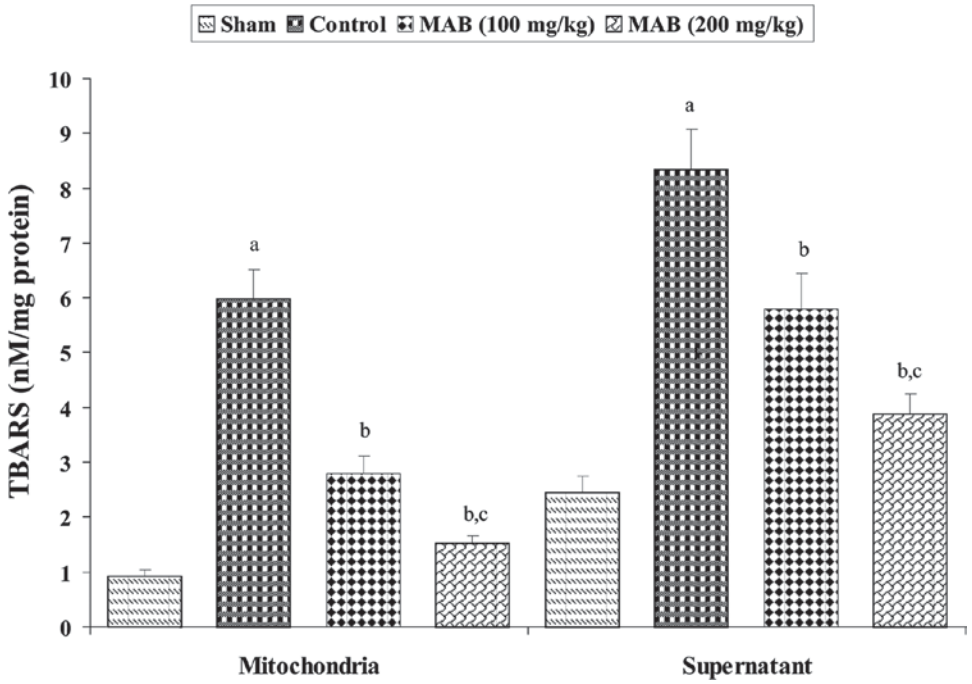

Figure 3. Effect of methanol extract of *Artemisia absinthium* (MAB) on mitochondrial and supernatant thiobarbituric acid reactive substances formation in mice subject to global cerebral ischemia followed by reperfusion. Each column represents the mean ± SD, $n = 7$; a = $p < 0.05$ versus sham; b = $p < 0.05$ versus control; c = $p < 0.05$ versus 100 mg/kg, p.o., of the extract. TBARS, thiobarbituric acid reactive substances.

Table 3. Effect of methanol extract of *Artemisia absinthium* (MAB) on superoxide dismutase (SOD) activity and reduced glutathione (GSH) content during cerebral post-ischemic reperfusion (15 min BCAO and 24-h reperfusion).

Group	SOD (U/mg protein)	GSH (nM/mg protein)
Sham	100.08 ± 14.11	5.59 ± 0.29
Control	63.50 ± 16.29**	2.24 ± 0.71**
MAB (100 mg/kg)	82.15 ± 7.45***	3.70 ± 0.45***
MAB (200 mg/kg)	95.99 ± 10.42**	5.60 ± 0.55**

All data are expressed as mean ± SD, $n = 7$. Sham and treatment groups are compared with control. Superscripts ** and *** indicate p values < 0.01 and < 0.05, respectively. Statistical analysis was done by one way analysis of variance followed by Tukey's Test.

cerebral injury (Reynolds et al., 2007). ROS produces malondialdehyde (MDA), an end product of lipid peroxidation. MDA reacts with TBA and is, thus, estimated as TBARS (Dib et al., 2002). Therefore, MDA was estimated using TBARS assay to estimate extent of ROS.

The over production of free-radicals can be detoxified by endogenous antioxidants causing their cellular stores to be depleted (Ahmad et al., 2006). GSH is considered a central component in the antioxidant defenses of cells. It acts both to directly detoxify ROS and as a substrate for various peroxidises. Moreover, it is well evidenced that SOD activity in serum is reduced in stroke patients, and replacement of antioxidant activity could be beneficial in the acute treatment of cerebral ischemia (Spranger et al., 1997). In the control groups, SOD and GSH activity was significantly reduced. Pre-treatment with MAB significantly prevented MCAO-induced decline in SOD and GSH activity.

A. absinthium has been reported to contain flavonoids (Zheng, 1994; Rice-Evans et al., 1996; Lee et al., 2004), thymol and carvacrol as well as other phenolic compounds (Kordali, et al., 2005). These pharmacophores have been shown to possess potent antioxidant and free-radical scavenging activity (Alessandra et al., 2003). In the present study, high levels of phenolic and flavonoid contents were estimated.

Conclusion

In conclusion, the present study indicates that *A. absinthium* possesses potent *in vitro* and *in vivo* antioxidant activity and free-radical scavenging capacity which could be due to the presence of phenols and flavonoids in the extract. These antioxidant activities could contribute, at least partly, to the therapeutic benefits of certain traditional claims of *A. absinthium*. In addition, MAB exhibited neuroprotection as is evident from the reduction of lipid peroxidation (decreased level of TBARS) and restoration of endogenous antioxidant (GSH and SOD) system. This suggests *A. absinthium* may be used as protective agent against disorders associated with oxidative stress.

Declaration of interest

The authors are grateful to the L.L.R. Educational Trust, which runs the L.R. Institute of Pharmacy, Solan, Himachal Pradesh, India, for providing funds to carry out the present investigation. The authors alone are responsible for the content and writing of the paper.

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