Original Article

Antioxidant potential of *Viscum articulatum* burm

Abstract

Background: Free radical stress leads to tissue injury and progression of disease conditions such as arthritis, hemorrhagic shock, atherosclerosis, diabetes, hepatic injury, aging and ischemia, reperfusion injury of many tissues, gastritis, tumor promotion, neurodegenerative diseases, and carcinogenesis. Safer antioxidants suitable for long term use are needed to prevent or stop the progression of free radical mediated disorders. *Viscum articulatum* is traditionally used for various oxidative stress induced disorders including liver disorders. **Aims:** The present study investigated antioxidant activities of the methanolic extract of *Viscum articulatum* in *in vivo* and *in vitro* models to provide scientific basis for the traditional usage of this plant. **Materials and Methods:** The *in vitro* antioxidant activity was evaluated by determining the ability of the extract to scavenge 2, 2-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide, 2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and hydrogen peroxide (H_2O_2) which were assessed using spectroscopic methods. **Results:** The extract showed promising dose dependant free radical scavenging property in all the methods used. The extract effectively increased the superoxide dismutase and catalase activity and decreased lipid peroxidation in the treated groups indicating *in vivo* antioxidant activity. The extract also effectively decreased the serum levels of SGOT, SGPT, SALP, and total protein levels compared to toxicant control rats. **Conclusion:** The results obtained from this study indicate that *Viscum articulatum* is a potential source of antioxidant which would help in preventing many free radical mediated diseases.

Key words:
ABTS, ABTS scavenging, antioxidant, catalase, DPPH, lipid peroxidation, nitric oxide, superoxide dismutase, Viscum articulatum

Introduction

Many herbs contain antioxidant compounds which protects cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrite.[1,2] Antioxidants that are mainly supplied as dietary consumptions can impede carcinogenesis by scavenging oxygen radicals or interfering with the binding of carcinogens to DNA. This includes vitamin C, vitamin E, carotenoids (cryptoxanthin, lutein, zeaxanthin, lycopene), and several polyphenolic compounds including flavonoids (catechins, flavonols, flavones, isoflavonoids).[3,4] In particular, phenolic compounds have been reported to be associated with antioxidative action in biological systems by acting as scavengers of singlet oxygen and free radicals.[5] The antioxidative potential of phenolic compounds can be attributed to their strong capability to transfer electrons to ROS/free radicals, chelating metal ions, activating antioxidant enzymes, and inhibitory oxidases.[6] In addition, free radicals and other reactive species are constantly generated *in vivo* and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged lipids and proteins.[7] Plant products are widely used in testing because of their low toxicity and great medicinal value. Much research has focused on the abilities of plant extracts to induce antioxidant effects.

Viscum articulatum of family Viscaceae has been traditionally used in conditions like Bacillary dysentery, Bronchitis, Fever, Lactation Deficiency, Lumbar Muscle Strain, Psoriasis,

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Kannoth Mukundan Geetha, Venkatesh Bindu, Vedigounder Murugan

Department of Pharmacology, Dayananda Sagar College of Pharmacy, Bangalore, Karnataka, India

Address for correspondence:
Dr. Kannoth Mukundan Geetha,
Dayananda Sagar College of Pharmacy, Kumaraswamy Layout,
Bangalore, Karnataka, India.
E-mail: geethakm@yahoo.com

Rheumatoid arthritis, Syphilis, Tuberculosis, and Urinary Tract Infections. The present work was aimed to evaluate the antioxidant activity of methonolic extract of *Viscum articulatum* (MEVA) by both *in vitro* and *in vivo* methods.

Materials and Methods

Plant material

It was obtained from Tirumala Hills, Thirupati, India. The plant was identified, confirmed, and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh.

Plant extract

The stem and branches were cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder using a mechanical grinder. The resulting powder was dried and packed well in Soxhlet apparatus and extracted with 1500 ml of methanol for 7 days. The extract was concentrated and dried using Rotary flash evaporator.

In vitro antioxidant studies

MEVA was tested for its free radical scavenging property using different *in vitro* models. All experiments were performed thrice and results were averaged.

DPPH assay

To 1 ml of various concentrations of MEVA (2, 4, 8, 16, 32, 64, 128, 256, 512 and 1000 μg/ml) 1 ml solution of DPPH, 0.1 mM was added. An equal amount of water and DPPH was added and was used as control. Ascorbic acid was used as standard for comparison. After incubation for 20 min in dark, absorbance was recorded at 517 nm.[8] The % inhibition of DPPH radical was calculated by comparing the results of the test with those of the control using the formula

%Scavenging = OD of Control – OD of Test/OD of Control × 100

Hydrogen peroxide assay

To 1 ml of various concentrations of MEVA, 2 ml solution of hydrogen peroxide (20 mM was prepared in phosphate buffer) was added. After incubation for 10 min in dark, absorbance was recorded at 230 nm.[9] Methanol was used as blank. Ascorbic acid was used as standard for comparison. The % inhibition of hydrogen peroxide radical was calculated by comparing the results of the test with those of the control using the standard formula.

Nitric oxide scavenging assay

To 0.5 ml of various concentrations of MEVA, 2 ml of sodium nitropruside solution and 0.5 ml of phosphate buffer was added. The reaction mixture was incubated at 25°C for 180 min. To 0.5 ml of incubated reaction mixture 1 ml of sulphanilic acid was added and allowed to stand for 5 min. One milliliter of NEDD was added and incubated for 30 min. After incubation absorbance was measured at 540 nm.[10] Ascorbic acid was used as standard for comparison. The % inhibition of nitric oxide radical was calculated by comparing the results of the test with those of the control using the standard formula.

ABTS assay

To 0.5 ml of various concentrations of extract, 0.3 ml of ABTS radical cation in potassium persulphate and 1.7 ml of phosphate buffer was added. Ascorbic acid was used as standard for comparison. Absorbance was measured at 734 nm.% scavenging was calculated.[11]

In vivo antioxidant activity

Albino rats (Wistar strain) of either sex weighing between 150 and 200 g were procured from the Sri Raghavendra enterprises, Bangalore, Karnataka. The animals were acclimatized for 7 days under laboratory conditions. The animals were fed with commercially available rat pelleted diet. Water was allowed *ad libitum* under strict hygienic conditions. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) bearing approval No. DSCP/MPharm.Col/IAEC/30/10-11.

Experimental design

Animals were divided into six groups of six animals each. Group-I animals served as normal control and was treated with 1% CMC, Group-II animals served as disease control and was treated with CCl_4 at a dose of 1 ml/kg b.w p.o to produce oxidative stress, Group-III served as standard, and was administered with Vitamin E at a dose of 50 mg/kg b.w p.o. Group IV, V, and VI were administered with daily doses of 100, 200, and 400 mg/kg b.w p.o of MEVA, respectively, for 14 days. Animals of groups III-VI were administered with CCl_4 at a dose of 1 ml/kg b.w p.o daily, half an hour prior to the administration of the drugs.

The animals were kept starved overnight on the 14th day of experiment. On the next day, the same animals were anesthetized using anesthetic ether and 2 ml of blood collected from retro orbital puncture. The serum was separated and used for estimation of biochemical parameters like ALT, AST, and total proteins. The animals were killed by overdose of ether and autopsed. The liver was rapidly excised, rinsed in ice cold saline, and a 10% w/v homogenate was prepared using 0.15 M KCl and centrifuged at 2000 rpm for 10 min. The supernatant was used for estimation of antioxidant parameters like Catalase, Superoxide Dismutase, and Thio barbituratic acid reactive substances.

Antioxidant Parameters

Estimation of catalase activity

A total of 100 μl of the tissue homogenate was added to 2.25 ml of 65 mM potassium phosphate buffer and 0.65 ml of 7.5 mM H_2O_2 was added and thoroughly mixed. The

rate of changes in the absorbance at 240 nm for 2-3 min was recorded. Catalase activity was expressed as unit/mg protein.[12]

Superoxide dismutase activity

To 0.05 M sodium carbonate buffer solution, 0.1 ml of tissue homogenate was added and incubated at 30°C for 45 min. Absorbance was recorded at 480 nm, and the absorbance obtained was adjusted to 0, and reaction was initiated by addition of 10 µl of adrenaline solution. The change in absorbance was recorded at 480 nm for 8-12 min. Throughout the assay, the temperature was maintained at 30°C. SOD activity was expressed as unit/mg protein.[13]

Determination of lipid peroxidation

To 1 ml of tissue homogenate, 0.2 ml of sodium lauryl sulfate solution, 1.5 ml of 20% acetic acid and 1.5 ml of thiobarbituric acid solution were added. This incubation mixture was made upto 5.0 ml with double distilled water and then heated in boiling water bath for 30 min. After cooling, the red chromogen was extracted with 5 ml of the mixture of n-butanol and pyridine and centrifuged at 4000 rpm for 10 min. The organic layer was taken and its absorbance was measured at 532 nm. The results were expressed as nM of MDA/mg of wet tissue using molar extinction co-efficient of the chromophore, (1.56×10^5 $mmol^{-1}$ cm^{-1}) Lipid peroxidation was expressed as nmol MDA/min/mg protein.[14]

Statistical analysis

Graphical method was used to calculate IC_{50} values wherever needed. All the results are shown as average±S.E.M. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by post Dunnett's multiple comparison test using Instat software. $P<0.05$ was considered as statistically significant.

Results

In vitro antioxidant activity

The IC_{50} values for MEVA and ascorbic acid were 8.8 and 4 µg/ml, respectively, by the DPPH method. MEVA showed increase in hydrogen peroxide scavenging activity with corresponding increase in its concentration with IC_{50} value of 2.2 µg/ml which was comparable to the IC_{50} value of ascorbic acid (8.8 µg/ml).

MEVA effectively reduced the generation of nitric oxide from sodium nitropruside. The extract showed nitric oxide scavenging activity with an IC_{50} of 16 µg/ml in comparison to standard ascorbic acid with an IC_{50} of 2.2 µg/ml. MEVA decreases the amount of nitrite generated from the decomposition of sodium nitropruside *in vitro*.

MEVA effectively reduces ABTS radical. The extract showed free radical scavenging activity with IC_{50} value of 51.2 µg/ml, whereas ascorbic acid exhibited an IC_{50} of 3.2 µg/ml.

In vivo antioxidant activity

Biochemical parameters (SGOT, SGPT, SALP, total protein) are shown in Table 1. The level of SGPT, SGOT, SALP, total protein is restored toward the normal value in MEVA treated carbon tetrachloride intoxicated rats. It was found from Table 1 that in CCl_4 control group showed a substantial increase in the level of serum marker enzymes (SGOT, SGPT, and SALP). The rise in protein levels in the treated groups suggests the stabilization of endoplasmic reticulum leading to protein synthesis.

Estimation of antioxidant parameters: Catalase, superoxide dismutase, lipid peroxidation

CAT, SOD, and lipid peroxidation (LPO) levels of liver homogenate were estimated and are summarized in Table 2. The level of CAT and SOD were reduced and LPO levels were increased in CCl_4 treated groups. However, CAT and SOD levels increased and LPO levels decreased toward the normal value in MEVA and vitamin E treated carbon tetrachloride intoxicated rats. The level of MDA, which is one of the end products of lipid peroxidation in liver tissue, was found to be high in CCl_4 control group [Table 2] implying enhanced lipid peroxidation.

Table 1: Effect of methanolic extract of *Viscum articulatum* on serum enzyme levels and total protein in CCl_4 intoxicated rats

Treated group	SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Total protein (mg/dL)
Carbon tetrachloride	92.87±10.12**	96.57±9.44**	92.95±4.02**	3.81±0.38**
Normal control group	39.299±1.26	60.35±1.84	33.0±1.12	5.98±0.708
Vitamin E (standard)	50.65±1.66*	62.48±1.98*	40.05±1.19*	5.87±0.649*
MEVA (100 mg/kg)	79.08±1.518*	78.81±6.33*	80.02±2.98*	4.52±0.447*
MEVA (200 mg/kg)	97.76±1.40*	62.32±5.93	52.75±3.28*	5.57±0.415*
MEVA (400 mg/kg)	64.54±1.44*	65.57±6.53	50.36±2.78*	5.25±0.428*

Values are mean±S.E.M.; $n=6$ in each group. Drug treatment was done for 14 days; **$P<0.001$ CCl_4 treated group compared with normal group; *Represents significant at $P<0.05$; **Represents highly significant at $P<0.01$, where the statistical analysis was performed by one way ANOVA followed by post Dunnett's multiple comparison test; SGOT – Serum glutamate oxaloacetate transaminase; SGPT – Serum glutamate pyruvate transaminase; SALP – Serum alkaline phosphatase; MEVA - Methanolic extract of *Viscum articulatum*

Table 2: Effect of methanolic extract of *Viscum articulatum* on lipid peroxidation, superoxide dismutase and catalase levels in CCl_4 intoxicated rats

Treated group	Lipid peroxidation [MDA content (in nano mole/milligram)]	CAT (μM of H_2O_2 decomposed/min/mg wet tissue)	SOD (U/mg wet tissue)
Carbon tetrachloride	463.3±5.59*	16.75±0.0250#	1.020±0.62#
Normal	301.65±0.757	25.61±0.0434	2.793±0.9.6
Vitamin E (standard)	361.4±4.0398*	21.76±0.013*	2.107±0.92*
MEVA (100 mg/kg)	373.4±7.35*	22.08±0.007*	2.474±0.32*
MEVA (200 mg/kg)	355.7±7.59*	23.03±0.065*	2.562±0.35*
MEVA (400 mg/kg)	396.9±7.10*	21.03±0.037*	1.979±0.42*

Values are mean±S.E.M; n=6 in each group. Drug treatment was done for 14 days; #P<0.001, CCl4 treated group compared with normal group; *Represents significant at P<0.05; where the statistical analysis was performed by one way ANOVA followed by post Dunnett's multiple comparison test; CAT – Catalase; SOD – Superoxide dismutase; MEVA – Methanolic extract of *Viscum articulatum*

Treatment with MEVA, vitamin E significantly reversed these changes.

Discussion

Free radicals are known to play a definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, Alzheimer, hepatic damage etc.[15] Antioxidants exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. The extract under study exhibited moderate antioxidant activity in the DPPH, H_2O_2, ABTS, and N_2O methods. This activity can be attributed to the presence of phenolic compounds present in the extract which have been reported to possess free radical scavenging activities.

In most of the developing countries, the incidence of diseases like cancer, diabetes, Alzheimer, viral hepatitis etc., is more, for which oxidative stress is the main reason. So, the investigation for an efficient antioxidant drug from the natural resource is an urgent necessity. The changes associated with CCl_4-induced oxidative stress are similar to that of manifestations in true oxidative stressed conditions. CCl_4 is therefore an useful tool for the induction of oxidative stress in experimental animals. The ability of antioxidant activity of MEVA to reduce the injurious effects or to preserve the normal physiological mechanisms that have been disturbed by an oxidative stress inducing agent is the index of its protective effects.

Conclusions

Present investigation suggests that MEVA shows good antioxidant activity. Phytochemical screening of the crude MEVA reveals the presence of flavonoids, saponins, phenolic compounds, tannins, steroids etc., Thus, these *in vitro* and *in vivo* antioxidant potentials of MEVA may be due to the presence of these phytoconstituents.

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