Research Article

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SCREENING OF FREE RADICAL SCAVENGING ACTIVITY OF AROGYAVARDHINI VATI

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Received on: 12/05/13 Revised on: 21/06/13 Accepted on: 10/07/13

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DOI: 10.7897/2277-4343.04421

Published by Moksha Publishing House. Website www.mokshaph.com

ABSTRACT

Free radicals are the main cause for the pathogenesis of disease. Considering present day's lifestyle oxidant stress is more on everyone. So anti-oxidants are in much demand worldwide. Only Ayurveda is science of life which has provided a rational basis for such thoughts and active medicines. Arogyavardhini vati is a potent formulation explained in rasashastra for maintaining health. An attempt was made to establish scientific evidence for anti-oxidant property of Arogyvardhini vati. Oxidative stress was induced in albino rats with carbon tetra chloride in all the groups except control. In control group only oxidative stress was induced without drugs. In test group three different concentration of Arogyvardhini vati (10 mg / ml, 20 mg / ml and 50 mg / ml) was administered. In standard group vitamin-c was used. Lipid peroxidation, Glutathione, Catalase Amylase, superoxide dismutase levels were estimated for four days. In an antioxidant assay Arogyvardhini vati 10 mg / ml and 20 mg / ml showed the significant reduction of malondialdehyde concentration and significant improvement in Glutathione, superoxide dismutase, Catalase Amylase activity. Antioxidant activity of Arogyvardhini vati was comparable to that of standard. Arogyvardhini vati demonstrated significant anti-oxidant activity compared to standard and control. This scientific data has given further scope for study in a larger samples and clinical study.

Keywords: Arogyavardhini vati, anti-oxidant, TBARS, vitamin-C.

INTRODUCTION

Oxidation of body cells can lead to generation of free radicals that are solely responsible for aging, cancer, atherosclerosis as well as a degenerative process happening in the body.[1] Reactive oxygen species are main causes for the pathogenesis of disease. Considering the present days lifestyle and food habits 'oxidant stress' is more on an individual. Same is responsible for exposure of an individual to various diseases.[2, 3] As natural antioxidants from herbal source provide protection that slows down the process of oxidative damage caused by free radicles.[4] Ayurveda sciences is based on biophysical, biochemical, physiological and biopharmacodynamic principles. 'Rasa chikitsa' is considered as 'daivee chikitsa' in Ayurveda. Rasayanas are the groups herbal, herbo-mineral and herbo-metalic preparations explained in Ayurvedic classics to prevent the process of aging. These will help for the nourishment of dhatus from rasa to shukra ultimately the ojas, which is responsible for physical, psycho-intellectual performances and immunity. Thus rasayana promotes long span of youthful life with full of vigor and free from diseases. It prevents the adverse effects of ageing and therefore, it can be postulated that rasayana drugs may have free radical scavenging property.[5,6] Arogyavardhini vati is one of the potent formulations explained in Rasaratna samuchchaya[7]. It is having the properties like kusthaghna, jwaraghna, hrudya, medo vinashana, pathya and is most successfully used in kamala and prameha. Contents of Arogyvardhini vati improve digestion, metabolism and other vital functions of the body. It nourishes the dhatus from rasa to shukra finally ojus. Arogyvardhini vati regenerates the degenerated cells of a body and thus is expected to have anti-oxidant activity, hence the named 'Arogyavardhini vati' (Health promoter). In the present study, screening of free radical scavenging activity of Arogyvardhini vati was under taken to provide a scientific data.

MATERIALS AND METHODS

Arogyvardhini vati, rat liver homogenates, Carbon tetrachloride, tragacanth, Malondialdehyde solution, vitamin C (Standard) formed the material for the study. Solvent used for extraction of materials and anti-oxidant study were of analytical grade.

Preparation of Arogyavardhini vati

Arogyvardhini vati was prepared by standard method described in Ayurvedic classical text[7]. Kajjali (black mercuric sulphide) (20 g), Loha bhasma (Incinerated iron) (10 g), Abhraka bhasma (Incinirated mica) (10 g), Tamra bhasma (Incinerated Copper) (10 g), Haritaki (*Terminalia chebula*) (6.6 g), Amalaki (*Emblica officinalis*) (6.6 g), Bibhitaki (*Terminalia belarica*) (6.6 g), Suddha shilajatu (black bitmin) (30 g), Chitraka moola (*Plumbago zylanica*) (40 g), Shuddha guggulu (*Chomiphera mukul*) (40 g), Katuki (*Picrorhiza kurroa*) (180 g) were weighed accurately. First kajjali and bhasmas were placed in khalwa and mixed properly. To it remaining powders were added and mixed thoroughly. Nimba patra swarasa was added till the mixture immersed completely (Samyagpluta) and trituration was carried out till mixture

get into semisolid form and dried. Same procedure was followed for second bhavana also and pills were made. The pills were dried and stored in glass bottle.

Preparation of Compound Powder of Tragacant (CPT)

CPT was prepared by mixing tragacanth, gum acacia, starch (50 g each) and sucrose (150 g) which was used as a drug vector.

Preparation of Rat Liver Homogenate

A solution of 30 % rat liver homogenate in ice cold KCl (0.15 M) was prepared by using homogeniser and 0.5 ml of the homogenates was transferred to small conical flasks.

Effect on Antioxidant Enzymes

The test was divided into 6 groups. In the first group, which served as control, only drug vector was added. While to the second group carbon tetrachloride (40 µl) was added along with drug vector. Test group consists of three different doses, where along with Arogyvardhini vati (10 mg/ml, 20 mg/ml and 50 mg/ml) carbon tetrachloride (40 µl) was added, vitamin C (5.5 mg/ml) as standard with carbon tetra chloride (40 µl) was added. To all the test groups' drug vector was added. Levels of antioxidant enzymes were assayed on 1st, 2nd and 4th day.

Estimation of Serum Lipid Peroxide

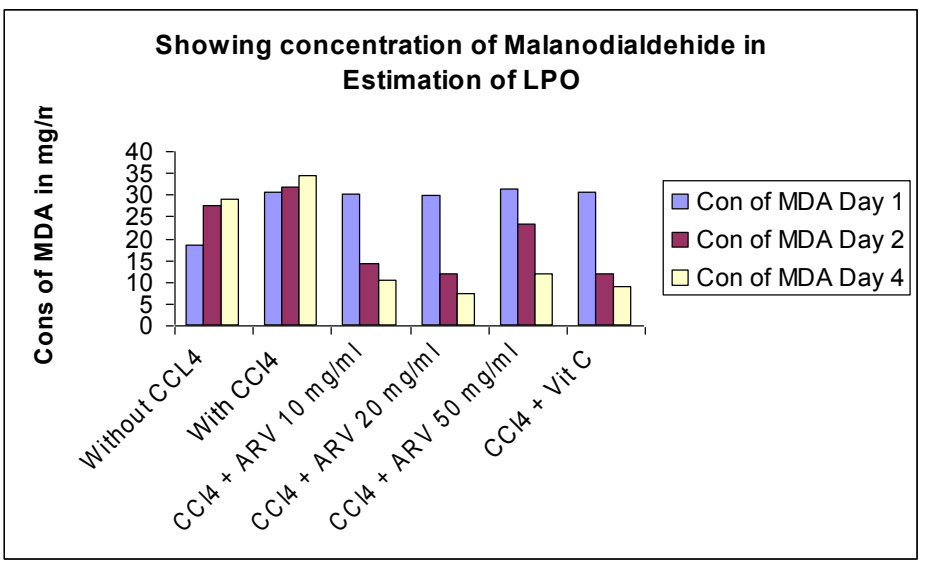
The amount of lipid peroxidation products present in serum samples were estimated by thiobarbuituric acid (TBA) method.[8] Lipid peroxidation in the liver homogenate was concluded by measuring the amounts of malondialdehyde solution concentration. To 0.5 ml of tissue homogenate, 4 ml of 10 % trichloroacetic acid was added and the content was centrifuged at 4000 rpm. To the supernatant, 1.5 ml of 0.67 % thiobarbituric acid was added, heated for 1 h on water bath and cooled to room temperature and final volume was made to 5.0 ml in each tube. The degree of lipid peroxides in homogenate was noted as absorbance at 540 nm in an UV spectrophotometer.

Estimation of Superoxide Dismutase

Ability to inhibit the autoxidation of pyrogallol by enzyme superoxide dismutase (superoxide dismutase)[9] was studied by adding 0.25 ml of absolute ethanol and 0.15 ml of 0.25 M sucrose solution to 2 ml of the homogenate. After 15 minutes of shaking in a mechanical shaker, the suspension was centrifuged at 4000 rpm for 15 minutes. To 50 µL of the supernatant, 3 ml of triscacodylate buffer (50 mM, pH 8.2) containing air equilibrated 0.2 mM pyrogallol, 1 mM ethylyne diamine tetra acetate, 1 mM diethylene triamine penta acetic acid were added. The rate of increase in the absorbance at 420 nm was recorded for 2 minutes, from 1 minute 30 sec to 3 minutes 30 sec in a spectrophotometer. The inhibition of pyrogallol by superoxide dismutase was measured at 420 nm and the activity expressed as units / mg protein.

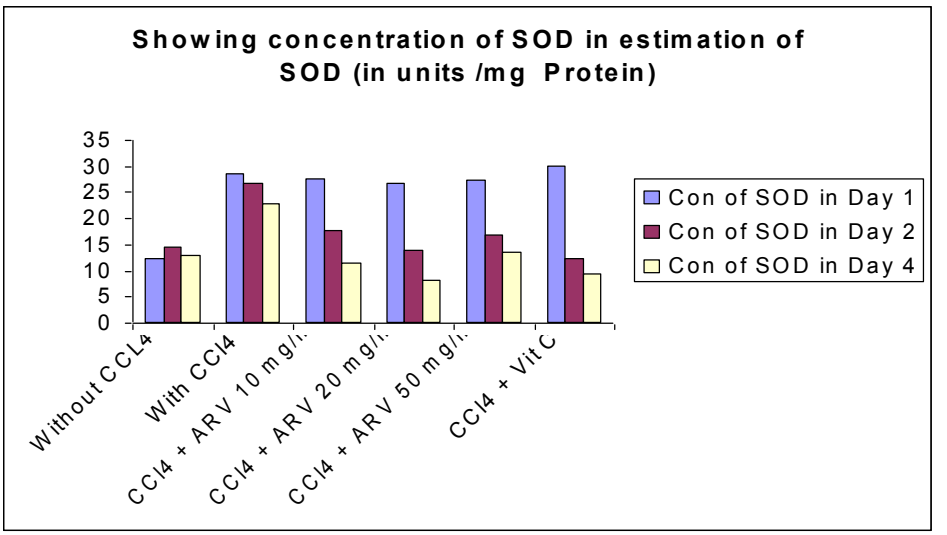
Estimation of Glutathione Peroxidase

It is based on reaction between thiol reagent, 5-5'-dithiobis[2-nitrobenzoic acid] with glutathione to form chromophore, 5-thionitrobenzoic acid and Glutathione trinitro benzoic acid[10]. To 0.2 ml of incubation homogenate was transferred to a tube containing 1.8 ml of precipitating buffer (5 % trichloro acetic acid in 1 mM ethylyne diamine tetra acetate). The sample was centrifuged at 2000 g for 10 minutes and supernatant was collected. To 2.0 ml of supernatant liquid, 4.0 ml of 0.3 M diSuperoxide dismutaseium hydrogen phosphate buffer (pH 8.0) was mixed and colour was developed by adding 0.01 % of 100 ml 5,5–dithiobis (2-nitrobenzoic acid). Absorbance was determined at 412 nm using spectrophotometer. The glutathione values are expressed as units / mg protein.

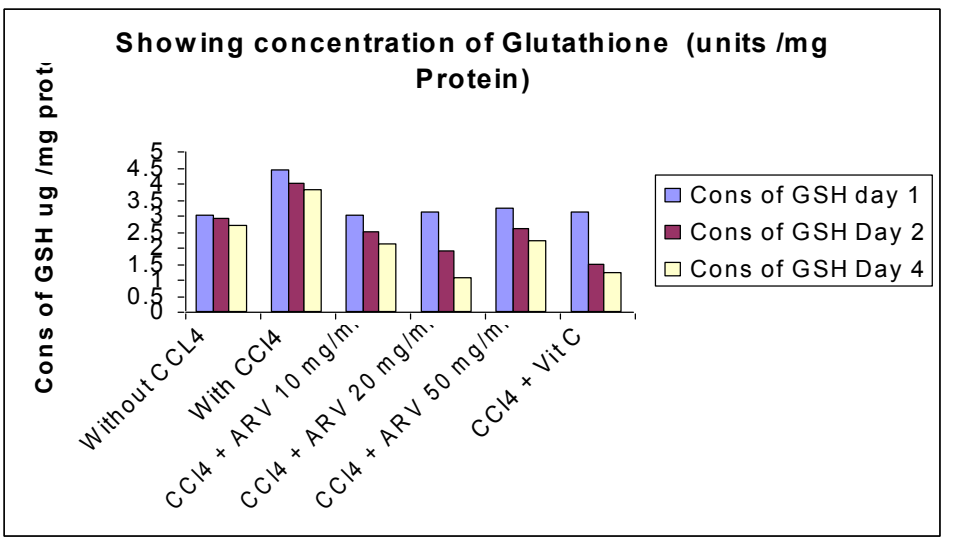


Graph 1: Concentration of Malondialdehyde in Estimation of LPO

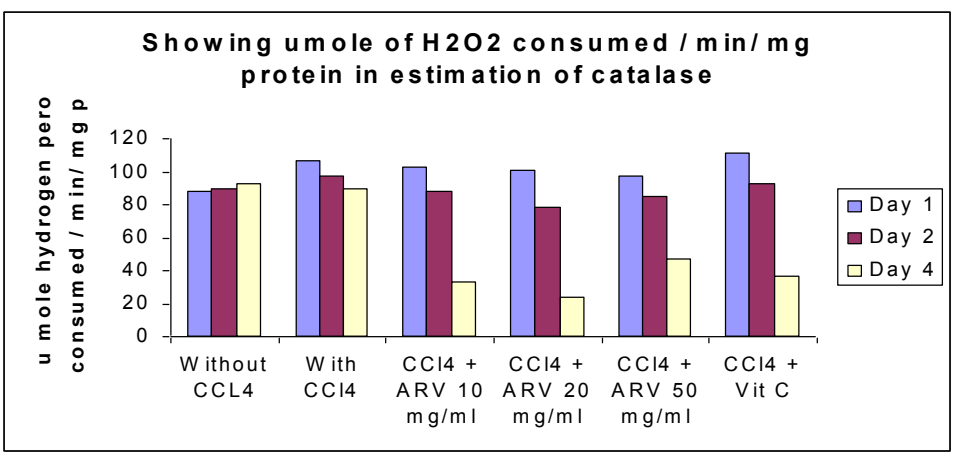
ARV = Arogyavardhini vati, CCL4 = Carbon tetra chloride, LPO = lipid peroxide, MDA = Malondialdehyde



Graph 2: Concentration of Superoxide Dismutase in Estimation of Superoxide Dismutase (In units / mg protein)
ARV = Arogyavardhini vati CCL4 = Carbon tetra chloride SOD = superoxide dismutase



Graph 3: Concentration of Glutathione (units / mg protein)
ARV = Arogyavardhini vati CCL_4 = Carbon tetra chloride GSH = Glutathione



Graph 4: µmole of H_2O_2 Consumed / min / mg Protein in Estimation of Catalase Amylase
ARV = Arogyavardhini vati CCL4 = Carbon tetra chloride

Estimation of Catalase Amylase

Catalase Amylase activity was determined by following reduction of hydrogen peroxide[11]. To 10 µl of the homogenate, 5 ml of Phosphate buffer (65 mM, pH 7.8) and 0.3 ml of hydrogen peroxide (7.5 mM) was added and centrifuged at 4000 rpm. Using the supernatant liquid, Catalase Amylase activity was measured spectrophotometrically at 240 nm and one unit activity was defined as the amount of enzymes, which liberates half the peroxide oxygen from a hydrogen peroxide solution per minute. Catalase Amylase activity is expressed as µmole of H_2O_2 consumed/min/mg.[12]

RESULTS

Oxidative stress was induced with Carbon tetra chloride in all the groups except control group. It was found that in all the groups (Control, standard and test groups) concentration of malondialdehyde was nearly similar on first day. This shows 40 µl of Carbon tetra chloride was sufficient to induce oxidative stress and effect of drug was not seen on first day.

Lipid peroxidation

In case of lipid peroxidation the concentration of malondialdehyde was found to be increased in homogenate treated with only Carbon tetrachloride i.e 30.59 nM/ltr, 31.71 nM/ltr, 34.12 nM/ltr on 1st, 2nd and 4th day respectively This is because of oxidative stress induced by Carbon tetra chloride and lack of protective agents against oxidation. On second and fourth day Arogyvardhini vati suspension 10 mg/ml (14.25 nM/ltr and 10.11 nM/ltr), 20 mg/ml (11.77 nM/ltr and 7.08 nM/ltr) and 50 mg / ml (23.28 nM/ltr and 11.71 nM/ltr) reduced concentration of malondialdehyde significantly. 20 mg/ml Arogyvardhini vati on fourth day found significant i.e 7.08 nM/ltr than standard drug vitamin-C which reduced malondialdehyde to 8.880 nM/ltr.

Enzyme Superoxide dismutase

The activity of Superoxide dismutase was found to be less i.e. 28.3 units/mg protein, 26.5 units/mg protein, 22.6 units/mg protein in homogenate treated with only Carbon tetra chloride. It may be due to oxidative damage by Carbon tetrachloride. Arogyvardhini vati with concentration of 10 mg/ml, 20 mg/ml and 50 mg/ml significantly increased activity of superoxide dismutase on fourth day i.e. 10 mg/ml (11.3 units/mg protein), 20 mg/ml (8.1 units/mg protein) and 50 mg/ml (13.6 units/mg protein). 20 mg/ml solution of Arogyvardhini vati was found significant in superoxide dismutase activity (8.1 units/mg protein) than standard drug (9.3 units/mg protein) and 10 mg/ml, 50 mg/ml solution of Arogyvardhini vati.

Enzyme glutathione

The enzyme glutathione activity was significantly increased on fourth day with solution of Arogyvardhini vati i.e. 10 mg/ml (2.1 units/mg protein), 20 mg/ml (1.07 units/mg protein) and 50 mg/ml (2.2 units/mg protein) compared to control with only oxidative stress i.e. 3.8. Test drugs results were comparable with standard i.e. (3.12 units/mg protein, 1.5 units/mg protein and 1.2 units/mg protein). 20 mg/ml solution of Arogyvardhini vati was found significant in glutathione activity i.e. 1.07 units/mg protein than standard drug (1.2 units/mg protein) and 10 mg/ml, 50 mg/ml solution of Arogyvardhini vati.

Enzyme Catalase Amylase

Significant increase in enzyme Catalase Amylase activity on fourth day was noted with Arogyvardhini vati 10 mg/ml, 20 mg/ml and 50 mg/ml solutions i.e (32.67 units/mg protein), (23.4 units/mg protein) and (46.8 units/mg protein) respectively, compared to the control (89 units / mg protein). 20 mg/ml solution of Arogyvardhini vati was found significant in Catalase Amylase activity i.e. 23.44 units/mg protein than standard drug i.e. 35.92 units/mg protein and 10 mg/ml, 50 mg/ml solutions of Arogyvardhini vati.

CONCLUSION

Arogyvardhini vati potentiates the antioxidant activity in rat liver homogenate with four parameters. Rat liver homogenates treated with Arogyvardhini vati shown less degree of Carbon tetra chloride inducing hepatic damage. In case of lipid peroxidation, on first day, concentration of malondialdehyde was more in all the homogenate treated with only Carbon tetra chloride. This was reduced significantly in homogenate treated with Arogyvardhini vati. 20 mg/ml was significantly found in reducing concentration of malondialdehyde (7.08 nM/ltr) than standard drug vitamin-C (8.880 nM/ltr). A significant increase in superoxide dismutase and Catalase Amylase activity was noted with all concentrations of Arogyvardhini vati as compared to control treated with Carbon tetra chloride. 20 mg/ml of Arogyvardhini vati was significant in Catalase Amylase activity than standard drug. Activity of glutathione was significantly increased with Arogyvardhini vati than control (treated with only Carbon tetra chloride). Thus Arogyvardhini vati significantly increased activity of antioxidant enzymes Superoxide dismutase, glutathione and Catalase Amylase. 20 mg/ml concentration was found to be more effective. From the above results it is noted that Arogyvardhini vati has shown significant free radical scavenging activity compared to vitamin-C. As the significant results were seen in all the parameters, probable mechanism of action may be explained as Arogyvardhini vati suppress the formation of free radicals. Increased activity of superoxide dismutase, catalase, glutathione indicates dis proportionation of superoxide, hydrogen peroxide and free fatty acid hydroperoxides. Katuki, triphala, Shilajatu, Abhrak bhasma, Tamra bhasma and nimba swarasa used for bhavana. Saponins, flavones and carotenoids present in Arogyvardhini vati might have contributed for antioxidant activity. Worldwide debate is on for controversies in the use of Ayurvedic herbo-mineral preparations. This study becomes more relevant and contributes evidence based data for antioxidant activity of Arogyvardhini vati.

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Cite this article as:
Sarashetti R.S., Simpi C.C., Sandeep N.M., Kanthi V. G. Screening of free radical scavenging activity of Arogyavardhini vati. Int. J. Res. Ayurveda Pharm. 2013;4(4):555-559 http://dx.doi.org/10.7897/2277-4343.04421

Source of support: Nil, Conflict of interest: None Declared