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Scavenging Strategy of *Panax ginseng* Against Formed Free Radicals Under Stress of Mercuric Chloride in *Rattus norvegicus*

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Abstract : Twenty five albino rats were divided into five groups for conducting this experiment. The first group was for positive control (Vitamin C, ascorbic acid), the second group was of *Panax ginseng* (10 mg/kg body weight) treated group after bio-activity assay, the third group was of mercuric chloride treated group (0.033 mg/kg body weight) based on calculating LD_{50} 9.26 mg/kg body weight by probit analysis, the fourth group was of mercuric chloride (0.033 mg/kg body weight) followed by *Panax ginseng* (10 mg/kg body weight) and the fifth group was *Panax ginseng* (10 mg/kg body weight) followed by mercuric chloride (0.033 mg/kg body weight) treated group. The interval between intake of *Panax ginseng* and mercuric chloride was of 2 hours in groups, fourth and fifth respectively. Comparative free radical scavenging property of *Panax ginseng* was studied under three *in vitro* models (role model for calculating scavenging activity) viz. DPPH method (hydroxyl free radicals), Nitric oxide method (nitrile free radicals) and *Lipid peroxidation* (mercury free radicals).

Key words : Ascorbic acid, 1, 1 diphenyl 2-picryl hydrizide, thiobarbituric acid reactive substances, lipid peroxidation

INTRODUCTION

Increased generation of oxidative free radicals, or impaired antioxidant defense mechanisms, have been implicated in the ageing process, neurodegenerative conditions, including Parkinsonism and Alzheimer's disease, in chronic stress induced perturbed haemeostatis, including immuno depression, inflammation, diabetes mellitus, peptic ulcer and other diseased conditions[1]. Mercuric chloride is a serious pollutant and also increased generation of oxidative free radicals with toxic effects in living organism. Primary exposure occurs through environmental contamination as the result of mining, smelting, extensive industrial and agriculture usage including inhalation and ingestion via the food chain. Mercuric chloride enters an organism and exhibiting its toxicologic characteristic including neurotoxicity, nephrotoxicity[2] and gastrointestinal toxicity with ulceration and haemorrhage[3]. This oxidative status of an individual is balanced by the activity of non-enzymatic antioxidant (tocopherol, ascorbic acid and beta carotene) and oxidant tissue enzymes (super oxide dismutase, catalase and glutathione peroxidase) which together prevent reactive oxygen species formation but tissue oxidant enzymes mitigate the damage caused by cells at a slow pace. However, non-enzymatic oxidant compounds produced secondary toxicity like lipid peroxidation, liver injury, neurological disorder and head & neck cancer. On the other hand, *Panax ginseng* an herb belongs to *Araliaceae* family and having vast pharmacological activity with safe output.

Considering all these facts present study has been designed to find out scavenging strategy of *Panax ginseng* against formed free radicals under stress of mercuric chloride in *Rattus norvegicus*.

MATERIALS & METHODS

Experimental compound

Experimental compound (mercuric chloride) was obtained from Bayer India Ltd., Mumbai. The oral LD_{50} was determined on albino rats. The mercuric chloride was dissolved in distilled water and introduced orally by gavage tube. The data were analyzed by probit analysis for LD_{50} determination[4]. Rats from the control set were given distilled water only.

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Panax ginseng root powder was gifted by Prof. A. Kumar, Cancer and radiation biology laboratory, Department of Zoology, Rajasthan University, Jaipur (Rajasthan).

Experimental animals

Rattus norvegicus weighing approximately (120-130) gm of both the sexes were procured from inbreed colony and acclimatized to the laboratory condition for 2 weeks. They were housed in polypropylene cages at the 20±5°C temperature, 50±5% relative humidity and 12 hrs/day photoperiod. The animals were fed with a standard balanced diet (Hindustan Lever Ltd., Mumbai) and water was provided *ad libitum.*

Experimental protocol

Animals were divided into 5 groups. Group I (positive control) received ascorbic acid, group II received *Panax ginseng* (10 mg/kg b. wt), group III received mercuric chloride (0.033 mg/kg b. wt.) after LD_{50} (9.26 mg/kg b. wt.) determination, group IV received *Panax ginseng* followed by mercuric chloride while, group V received mercuric chloride followed by *Panax ginseng*. The gap in between *Panax ginseng* and mercuric chloride in group IV and V were 2 hours.

Preparation of rat liver homogenate

Randomly selected rats were fasted overnight. They were scarified by cervical dislocation, dissected and the whole liver was removed quickly. It was further processed to get 10% homogenate in 0.15 M KCl[5] using a Teflon homogenizer. The homogenate was filtered to get a clear solution and used as a source of polyunsaturated fatty acids for determining the extent of lipid peroxidation.

***In vitro* lipid peroxidation assay**

The extent of lipid peroxidation in rat liver homogenate was measured *in vitro* in terms of formation of thiobarbituric acid reactive substances (TBRAS). Different concentrations of the extract were made with ethanol. These samples were individually added to the liver homogenate (0.5 ml). This mixture was incubated with 0.15 M KCl (100 μl). Lipid peroxidation was initiated by adding 100 μl of 15 m M $FeSO_4$ solution. The reaction mixture was incubated at 37°C for 30 min. an equal volume of TBA: TCA (1:1, 1 ml) was added to the above solution followed by the addition of 1 ml BHT. This final mixture was heated on a water bath for 20 min at 80°C and cooled centrifuged and absorbance read at 532 nm[6] using a spectrophotometer (Cintra-5). The percentage inhibition of lipid peroxidation was calculated by comparing the results of the test with those of controls not treated with the extract as per the formula.

$$\text{Inhibition}(\%) = \frac{(\text{Control-Test})}{\text{Control}} \times 100$$

DPPH radical scavenging activity

DPPH scavenging activity was measured by the spectrophotometer[7]. To an ethanolic solution of DPPH (200 μM), 0.05 ml of the test compounds dissolved in ethanol were added at different concentrations. An equal amount of ethanol was added to the control. After 20 min the decrease in absorbance of the test mixtures (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated[7].

Scavenging of nitric oxide radical[8, 9]

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction as described previously[10, 11]. Sodium nitroprusside (5 m M) in standard phosphate buffer solution was incubated with different concentrations of the aqueous extract dissolved in phosphate buffer (0.025 M, pH: 7.4) and the tubes were incubated at 25°C for 5 hrs. Control experiments with out the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5 hrs, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess' reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrile with sulphailamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm. The experiment was repeated in triplicate.

RESULTS AND DISCUSSION

In DPPH method, scavenging percentage of *Panax ginseng* range from 43.70 to 28.23 at 250, 125, 63, 32, 16, 8, 4 and 2 μg/ml concentrations, while in ascorbic acid were from 79.79 to 4.20 at 64, 32, 16, 8, 4, 2, 1 and 0.5 μg/ml concentrations (Table 1). In nitric oxide method, scavenging percentage of *Panax ginseng* was 32.94 to 1.59, while it was 77.29 to 2.17 at the same concentration *vide supra* (Table 2). On the other hand *lipid peroxidation* were 40.07 to 1.25 in mercuric chloride treated set, 50.72 to 20.77 in *Panax ginseng* treated set, 60.33 to 22.33 in *Panax ginseng* followed by mercuric chloride set, while

Table 1. % inhibition by DPPH method

Concentration (μg/ml)	Ascorbic acid	Concentration (μg/ml)	Panax ginseng (root)
64	79.79±0.001	250	43.70±0.240
32	78.12±0.001	125	40.31±0.230
16	62.15±0.001	63	34.88±0.070
8	46.18±0.003	32	32.85±0.100
4	29.51±0.001	16	30.30±0.100
2	13.54±0.001	8	29.61±0.100
1	5.90±0.002	4	29.02±0.110
0.5	4.20±0.002	2	28.23±0.060
IC_{50}	10.77 μg/ml	IC_{50}	433.55 μg/ml

Table 2. % inhibition by Nitric oxide method

Concentration (μg/ml)	Ascorbic acid	Concentration (μg/ml)	Panax ginseng (root)
64	77.29±0.002	250	32.94±0.120
32	75.23±0.002	125	24.44±0.170
16	61.47±0.004	63	19.91±0.009
8	40.33±0.001	32	17.82±0.170
4	31.28±0.001	16	16.84±0.220
2	13.28±0.002	8	16.06±0.009
1	3.14±0.001	4	2.61±0.210
0.5	2.17±0.001	2	1.59±0.230
IC_{50}	49.78 μg/ml	IC_{50}	578.59 μg/ml

Table 3. % inhibition by lipid peroxidation method

Concentration (μg/ml)	Ascorbic acid	Concentration (μg/ml)	Mercuric chloride	Panax ginseng (root)	*Panax ginseng* followed by mercuric chloride	Mercuric chloride followed by *Panax ginseng*
64	69.66±0.140	250	40.07±1.110	50.72±0.290	60.33±0.880	45.33±0.330
32	66.69±0.130	125	32.68±1.400	40.18±0.110	45.33±0.330	36.33±0.670
16	60.50±0.009	63	20.70±1.330	35.06±0.020	34.67±0.670	23.33±0.330
8	48.21±0.510	32	15.70±1.230	24.33±0.130	30.67±0.330	18.33±0.330
4	36.40±0.210	16	6.61±1.110	23.10±0.020	28.66±0.880	8.33±0.330
2	25.00±0.390	8	4.57±0.580	22.12±0.020	26.67±0.880	6.67±0.330
1	16.00±0.190	4	3.06±0.680	21.76±0.210	24.33±0.670	4.33±0.670
0.5	12.00±0.007	2	1.25±0.790	20.77±0.290	22.33±0.330	2.33±0.330
IC_{50}	23.95 μg/ml	IC_{50}	321.70 μg/ml	248.75 μg/ml	225.52 μg/ml	360.82 μg/ml

43.33 to 2.33 in mercuric chloride followed by *Panax ginseng* at 250, 125, 63, 32, 16, 8, 4 and 2 μg/ml concentrations (Table 3). The calculated IC_{50} of *Panax ginseng*, ascorbic acid (In DPPH method), *Panax ginseng*, ascorbic acid (In Nitric oxide method), mercuric chloride, *Panax ginseng*, *Panax ginseng* followed by mercuric chloride, mercuric chloride followed by *Panax ginseng* and ascorbic acid (in *lipid peroxidation* method) were 433.55 μg/ml, 10.77 μg/ml, 578.59 μg/ml, 49.78 μg/ml, 321.70 μg/ml, 248.75 μg/ml, 225.52 μg/ml, 360.82 μg/ml and 23.95 respectively. The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl-perferyl complex[12] or through OH radical by Fenton reaction[13] there by initiating a cascade of oxidative reactions. The results obtained in the present studies may be attributed to several reasons viz. reducing the rate of conversions of ferrous to ferric or by chelation of the iron itself[14]. The moderate activity of the extract may probably due to the rapid and extensive degradation of the

antioxidant principles in an *ex vivo* state. It is also known that the · OH radical which initiates lipid peroxidation has a short life time (10^{-9} s at 37°C) and hence very difficult to investigate by conventional methods[15].

Nitric oxide is free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases[16, 17]. In the present study the nitrile produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25°C was reduced by the aqueous extract of *Panax ginseng*. This may be due to the antioxidant principles in the extract which complete with oxygen to react with nitric oxide[18] thereby inhibiting the generation of nitrile.

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. From the present results it may be postulated that *Panax ginseng* reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles[19]. DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses colour spectrophotometrically depending on the number of electron taken up[20]. The results showed that in DPPH method *Panax ginseng* inhibit the hydroxyl free radical; in nitric oxide method it inhibits nitrile free radicals, while in lipid peroxidation method it inhibits the mercury free radicals. The variations in all the three methods are due to free radical scavenging activity of ginsenosides and active constituents of *Panax ginseng*. The difference in IC_{50} at the same concentration also reveals scavenging activity of ginsenosides. Hence, it can be concluded that *Panax ginseng* has free radical scavenging activity against different free radicals under stress of mercuric chloride so it can be used as medicine in stressful condition.

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