IN VIVO ANTIOXIDANT ACTIVITY OF PLANT PINUS ROXBURGHII

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Summary

Objective of present work is to evaluate ethanolic extract of *Pinus roxburghii Sarg* bark and root for its potential in-vivo antioxidant potential against CCl_4 induced hepatotoxicity. Animals were treated with plants bark and root extract for seven days and then hepatotoxicity was induced with a single CCl_4 intraperitoneal injection. Pretreatment with 500 mg/kg (p.o.) of PRB (*P. roxburghii* bark extract) and PRR (*P. roxburghii* root extract) extract improved the Glutathione, SOD, Catalase, and Peroxidase levels significantly as compared to control group. The present studies revealed that *P. roxburghii Sarg* have significant in-vivo antioxidant activity.

Introduction

Pinus roxburghii Sarg. is the only tree with an ornamental specimen and having different medicinal values found in Himalayan region of Bhutan, Nepal, Kashmir, Sikkim, Tibet and other parts of north India[1] In the traditional system of medicine, there are several plants which are used in treating liver disorders. Their extract, fractions, and active constituents display marked hepatoprotective action, which has been related to their antioxidant properties. [2]

Free radicals are reactive molecules involved in many physiological processes and human diseases, such as cancer, aging, arthritis, Parkinson's syndrome, ischemia and liver injury. The elevation of free radical levels seen during the liver damage is owing to improved production of free radicals and decreased scavenging potential of the cells. Various intrinsic antioxidants (reduced Glutathione, Superoxide dismutase (SOD), Catalase and Peroxidase) are present in the organism, which protect them from oxidative stress, by it forming the first line of defense. [3] The present study was undertaken to evaluate antioxidant activity of alcoholic extract of roots and bark of *Pinus roxburghii Sarg* (PR) in rats.

Material and Method

Collection of plant material

The plant PR was collected from the Hilly region of Morni, Panchkula (Haryana), in the month of January 2009 and was authenticated by FRI, Dehradun, India. The dry powder of roots and bark of PR was extracted with 100% alcohol in a soxhlet apparatus for 24 h at approximately 60°C. The extract was then concentrated by distilling the solvent below 60°C and dried in a dessicator. A suspension was prepared by using equal volumes of the extract and gum acacia for administration to rats using oral gauge.

Chemicals used

The chemicals used for investigation of antioxidant activity were CCl_4 (Qualigens Ltd. India), Liv-52 (Himalaya, India), hydroxylamine hydrochloride (Sigma, India), nitro-blue tetrazoleum (NBT) (Sigma, India), hydrogen peroxide, EDTA, and Ellman's reagent (Sigma, India). All other chemicals bought from local sources were of analytical grade.

Animals

Albino rats of Wistar strain, weighing 100–150 g, kept on normal diet (Ashirwad industries, Ropar, Punjab) and water ad libitum, were divided into nine groups of six animals each. Before starting the experiment, permission from the Institutional Animal Ethics Committee was obtained.

Experimental

Group-I animals served as normal control, treated with distilled water. Group-II animals served as hepatotoxic control, treated with CCl_4 in a single dose of 1.5 ml/kg, i.p., to produce acute hepatotoxicity. Group III served as a standard group, and was administered Liv-52 in a dose of 56 mg/kg, p.o. Group-IV, V and -VI animals were treated with daily doses of 100, 300 and 500 mg/kg, p.o., respectively, of PR bark extract and Group-VII, VIII and -IX animals were treated with daily doses of 100, 300 and 500 mg/kg, p.o., respectively, of PR root extract for 7 days. The animals of Groups III–IX were given single dose of CCl_4, 1.5 ml/kg, i.p., 6 h after the last treatment.

On day 8 the rats were sacrificed by carotid bleeding and liver was rapidly excised, rinsed in ice-cold saline, and a 10% w/v homogenate was prepared using 0.15M KCI, centrifuged at 800 g for 10 min at 4°C. The supernatant obtained was used for the estimation of Catalase and Peroxidase. Further, the homogenate was centrifuged at 1000 g for 20 min at 4°C and the supernatant was used for biochemical estimation.

Biochemical estimation

Estimation of SOD

Estimation of SOD was done by autoxidation of hydroxylamine at pH 10.2, which was accompanied by reduction of NBT, and the nitrite produced in the presence of EDTA was detected colorimetrically. [4] One enzymatic unit of SOD is the amount in the form of proteins present in 100 µl of 10% liver homogenate required to inhibit the reduction of 24 mM NBT by 50% and is expressed as units per milligram of protein.

Estimation of Catalase

Catalase activity was estimated by determining the decomposition of H_2O_2 at 240 nm in an assay mixture containing phosphate buffer.[5] One international unit of Catalase utilized is that amount that catalyzes the decomposition of 1 mM H_2O_2/min/mg of protein at 37°C. Catalase activity was calculated using the millimolar extinction coefficient of 0.07 and expressed in terms of micromole per minute per milligram of protein.

Estimation of Peroxidase

Peroxidase estimation is based on periodide formation. Periodide can be spectrophotometrically determined at 353 nm, and this is directly proportional to the peroxidase concentration in the reaction mixture containing approximate amounts of H_2O_2 and enzyme. [6] One unit of peroxidase activity is defined as the change in absorbance per minute and expressed in terms of units per milligram of protein.

Estimation of Glutathione

Glutathione was estimated using Ellman's reagent (5, 5 dithiobis-(2-nitrobenzoic acid) [DTNB]). The sulphydryl groups present in glutathione forms a colored complex with DTNB, which was measured colorimetrically at 412 nm. The amount of glutathione was determined using its molar extinction coefficient of 13600/m/cm and expressed in terms of µmol/mg of protein [7].

Stastical Analysis

Results were subjected to one-way ANOVA. $P<0.05$ was considered significant. The post hoc analysis was carried out by Dunnet's multiple comparison test.

Results and Discussion

Table .1 Effect of Root and Bark extract on biochemical parameters

Treatment	Glutathione(µmol/mg of protein)	SOD(u/mg of protein)	Catalase(uM/min/mg of protein)	Peroxidase(u/mg of protein)
Normal control	18.5 ± 0.094 **	0.35±0.02	10±0.03	0.85±0.09
Hepatotoxic Control	8.4 ± 0.7	0.20±0.01	1.0±0.01	0.14±0.02
Standard Liv 52	16.3 ± 0.07**	0.30±0.01**	8.6±0.**	0.68±0.15**
PRB100 mg/kg	9.9 ± 0.06	0.24±0.02	1.7±0.2	0.16±0.06
PRB250 mg/kg	12.9 ± 0.1*	0.26±0.01	1.9±0.4	0.18±0.03
PRB500 mg/kg	15±0.9**	0.28±0.05*	5.0±0.4**	0.65±0.06**
PRR100 mg/kg	8.9 ± 0.06	0.27±0.01*	1.7±0.02	0.24±0.2
PRR 250 mg/kg	12.8 ± 0.1*	0.28±0.01*	2.1±0.3	0.5±0.03*
PRR 500 mg/kg	16±0.9**	0.29±0.03**	7.0±0.6**	0.67±0.13**

Values are in Mean±SEM. One-way. Where* $p<0.05$ and **$p<0.01$

As shown in Table 1, CCl_4 treatment decreased the level of SOD, catalase, and peroxidase whereas increased the level of Glutathione. Pretreatment with 500 mg/kg (p.o.) of PRB and PRR extract improved the Glutathione, SOD, Catalase and Peroxidase levels significantly. SOD is a ubiquitous cellular enzyme that dismutates superoxide radical to H_2O_2 and oxygen and is one of the chief cellular defence mechanisms. The H_2O_2 formed by SOD and other processes is scavenged by Catalase that catalyzes the dismutation of H_2O_2 into water and molecular oxygen. Thus, the antioxidant enzyme Catalase is responsible for detoxification of H_2O_2. Glutathione is a tripeptide of glycine, glutamic acid, and cysteine. The antioxidant enzymes form the first line of defence against free radical-induced damage, offer protection against free radicals, and thereby maintain low levels of lipid peroxide.[2] Peroxidase is an enzyme that catalyzes the reduction of hydroperoxides, including hydrogen peroxides, and functions to protect the cell from peroxidative damage. As the PRB and PRR extract, in the dose of 500 mg/kg, p.o., has improved the Glutathione, SOD, Catalase, and Peroxidase levels significantly, which were comparable with Liv 52. [3] We conclude that the alcoholic extract from the root and bark of PR possesses antioxidant activity, making it a plant to be used in treatment of liver disorders.

References

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