

ANTI OXIDANT PROPERTY METHANOLIC EXTRACT OF *Solanum pubescens* Willd., ON PARACETAMOL INDUCED OXIDATIVE STRESS IN ALBINO RATS

PEDDI SUMALATHA¹, K. HEMAMALINI², M. VIJUSHA³

Pharmacology, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad. Email: Sumapeddi39@gmail.com

Received: 01 Aug 2013, Revised and Accepted: 02 Sep 2013

ABSTRACT

Objective: To investigate antioxidant activity of methanolic leaf extract of *Solanum pubescens* Willd., on paracetamol induced oxidative stress in albino rats.

Methods: Paracetamol is administered as an inducer for oxidative stress. Thirty Wistar albino rats were randomly divided into five groups. Either sex of Wistar strains of albino rats were divided into 5 groups were Group 1 served as normal control, Group 2 served as Paracetamol control, Group 3 were administered with standard drug (Silymarin), Group 4 and Group 5 were administered to different doses of methanolic extract of *Solanum pubescens* Willd., (i.e. 200 and 300 mg/kg/Kg body weight) for 7 days. On the 7th day animals were sacrificed by cervical decapitation and the tissue homogenate was prepared to evaluate total protein and antioxidant activity were assayed by employing enzymatic and non enzymatic parameters from liver, such as Total protein, GSH, LPO, SOD, CAT, GSH peroxidase, Na⁺/K⁺ ATPase.

Results: Altered levels of the parameters were brought back to normal on treatment with methanolic extract of *Solanum pubescens* Willd.,

Conclusion: It was concluded from the result that the methanolic extract of *Solanum pubescens* Willd., showed significant antioxidant property in a dose dependent manner.

Keywords: *Solanum pubescens* Willd., Antioxidant activity, Oxidative stress, Paracetamol and Silymarin.

INTRODUCTION

Antioxidants

Oxidative stress, defined as "the imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to damage" has been suggested to be the cause of aging and various diseases in humans [1].

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against various infections and degenerative diseases [2]. Reactive oxygen species, such as superoxide anion radical (O₂⁻), hydrogen peroxide radical (H₂O₂) and hydroxyl radical are often generated as byproducts of biological reactions or from exogenous factors. The involvement of these species in the pathogenesis of a large number of diseases including rheumatoid arthritis, atherosclerosis, skin-aging, nephritis, reperfusion injury, asthma, diabetes mellitus and carcinogenesis are well documented [3]. A potent scavenger of these species may serve as a possible preventive intervention for free radical mediated diseases [4].

Solanum pubescens Willd., belongs to the family *Solanaceae*, have pharmacological actions like antidiabetic [5], hepatoprotective [6], gastroprotective [7], anti-inflammatory [8], antimicrobial [9], antihelmethic [10] and anti lice [11] activity and the secondary metabolites like flavonoids [12] which are responsible for those pharmacological activities are collected from literature review.

These flavonoids are responsible for free radical scavenging activity which is one of the main mechanisms of anti oxidant activity, no literature found on the antioxidant activity, So, we planned for a trial on anti oxidant property of methanolic extract of *Solanum pubescens* Willd.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Solanum pubescens* were collected from Chittoor district, Andhra Pradesh, India and authenticated by Dr. K. Madhava Chetty, Professor, Department of Botany S.V. University, Tirupathi, Andhra Pradesh, India.

Animals

Healthy albino rats of either sex weighing 150-200g were used for the present study. The animals were housed individually in polypropylene cages, maintained under standard conditions (12

hours light and 12 hours dark cycle, 25 + 5°C and 40-60% humidity). They were fed with standard rat pellet diet (National Institute for Nutrition, Hyderabad) and provided water ad libitum. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ethical committee IAEC Reg. No. (1447/PO/a/11/CPCSEA).

Antioxidant activity

Experimental Design

Paracetamol induced oxidative stress in albino rats [14]

Healthy albino rats of either sex weighing 150-200g were used as the experimental model, divided into 5 groups of 6 animals each. Group 1, served as control, receives only normal saline. Group-2 receives saline for 7 days and on 5th day paracetamol (2g/kg body weight) was administered. Group-3 receives standard drug silymarin (100mg/kg body weight) once daily for 7 days. On 5th day paracetamol 2g/kg was administered. Group 4, and 5 receives test drug in two different doses for 7 days and on 5th day after administration of herbal drug paracetamol 2g/kg was given orally.

Treatment protocol

Group 1: Receives saline (1ml/kg) for 7 days

Group 2: Receives saline for 7 days and on 5th day paracetamol 2g/kg was administered

Group 3: Receives silymarin (100mg/kg) for 7days. On 5th day Paracetamol 2g/kg was administered.

Group 4: Receives 200mg/kg of methanolic extract of *Solanum pubescens* once daily for 7 days and on 5th day after administration of herbal drug paracetamol 2 g/ kg was given orally.

Group 5: Receives 300mg/kg of methanolic extract of *Solanum pubescens* once daily for 7 days and on 5th day after administration of herbal drug Paracetamol 2 g/kg was given orally.

Biochemical studies

After treatment period, on the 7th day, the animals were sacrificed by cervical decapitation, blood was collected in clean dry test tubes and centrifuged at 3000rpm for 5 min. Serum was separated and used for estimation of total proteins. Liver was dissected out and washed in ice- cold saline followed by Tris-HCl (pH-4), blotted dry under air

and weighed with weighing balance. A 2g portion of the liver tissue was sliced and then homogenate after precipitation of protein using 10% TCA was used for the estimation of enzymatic and non enzymatic antioxidants.

Estimation of total protein [15]

The protein content of the tissue homogenate and serum were measured by Lowry method. 0.5ml of tissue homogenate & 0.5 ml of serum were mixed with 0.5ml of 10% TCA separately and centrifuged for 10 min. The precipitate obtained was dissolved in 1 ml of 0.1N NaOH, from this 0.1 ml used for the protein estimation.

Estimation Lipid Peroxidation (LPO) [16]

Reagents: 1.5ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetate buffer (pH3.5) and 1.5 ml of 0.8% TBA solution

As a marker for lipid peroxidation the levels of Thiobarbituric acid reactive substances (TBARS) in the liver was measured by the method of Ohkawa et al. A mixture of 0.4 ml of 10% of liver homogenate, 1.5ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetate buffer (pH3.5) and 1.5 ml of 0.8% TBA solution was heated at 95°C for 1h. After cooling, 5ml of n-butanol-pyridine (15:1) was added, and the absorbance of the n-butanol- pyridine layer was measured at 532 nm.

Estimation of glutathione (GSH)

Reagents: 10 % Tri chloro acetic acid (TCA), 1.8 ml of EDTA 0.6 mM 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) and 0.2 M 4.0 ml of disodium hydrogen phosphate solution pH 8.0

The GSH was determined by the method of Beutler and Kelly [17]. 0.2 ml of tissue homogenate was mixed with 1.8 ml of EDTA solution. To this 3.0 ml of precipitating reagent TCA was added mixed thoroughly and kept for 5min before centrifugation. To 2ml of filtrate, 4.0 ml of disodium hydrogen phosphate solution and 1.0 ml of DTNB (5, 5-dithio bis 2-nitro benzoic acid) reagent were added and the absorbance read at 412nm.

Assay of SOD [18]

Reagents: 1.2ml sodium pyrophosphate buffer (pH8.3, 0.025ml/ L), 0.1ml phenazine methosulphate (186m mol/L), 0.3ml Nitro blue tetrazolium (300n mol/L) and 0.2 ml NADH (780m mol/L).

The activity of SOD in tissue was assayed by the method of Kakkar . The assay mixture contained 1.2ml sodium pyrophosphate buffer (pH8.3, 0.025ml/ L), 0.1ml phenazine methosulphate (186m mol/L), 0.3ml Nitro blue tetrazolium (300n mol/L), 0.2 ml NADH (780m mol/L) and diluted enzyme preparation and water in a total volume of 3 ml. After incubation at 30°C for 90 sec, the reaction was terminated by the addition of 1.0ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml n-butanol. The chromogen in the butanol layer was measured at 560nm against n-butanol.

Assay of Catalase [19]

Reagents: Dichromate/acetic acid reagent (5 % solution of potassium dichromate in acetic acid at 1:3 ratio), 0.01 M Phosphate buffer, pH 7.0, 0.2 M Hydrogen peroxide.

Catalase (CAT) was estimated by the method of Sinha (1972). The reaction mixture (1.5 mL) contained 1.0 mL of 0.01 M phosphate buffer (pH 7.0) 0.1 mL of tissue homogenate and 0.4 mL of 2 M H₂O₂. The reaction was stopped by the addition of 2.0 mL dichromate-acetic acid reagent (5 % potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Then the absorbance was measured at 530 nm, CAT activity was expressed as μ M of H₂O₂ consumed/min/mg protein.

Assay of glutathione peroxidase [20]

Reagents: 0.2ml of glutathione, 0.1ml of hydrogen peroxide and 0.5ml of 10% TCA

The glutathione peroxidase activity was assayed according to the method of Rotruck. The assay homogenate. To this, mixture, 0.2ml of glutathione added followed by 0.1ml of hydrogen peroxide. The contents were mixed well and incubated at 37°C for 10 min. along with a control tubes containing all the reagents except tissue homogenate. After 10 min, the reaction was arrested by the addition of 0.5ml of 10% TCA. The tubes were assayed for Glutathione content by activity was expressed as mcg glutathione utilized /min/mg protein.

Assay of Na⁺/K⁺ ATPase [13]

Na⁺/K⁺ ATPase were assayed according to the procedure of Bonting. The assay mixture contained 1ml of the buffer, 0.2ml of each KCl, NaCl, EDTA and ATP solution and 0.2 ml of the tissue homogenate. The contents were incubated at 37°C for 15 min. At the end of the incubation period the reaction was arrested by the addition of 1ml of 10% TCA. The tubes were centrifuged and the phosphorous content of the supernatant was estimated according to Fiske and Subbarow's method. The enzyme activity was expressed as micromoles of Pi liberated/h/mg of protein.

Statistical Analysis

The mean value \pm SEM calculated for each parameter. Results were subjected to statistical analysis using Student t-test. The values were considered significant when P < 0.001.

RESULT AND DISCUSSION

Table 1: Effect of MESP on Total protein level on paracetamol induced oxidative stress in albino rats.

Group	Treatment	Total protein
1	Normal control (saline 2ml/kg)	6.480 \pm 0.064
2	Paracetamol 2g/kg	5.147 \pm 0.1145
3	Paracetamol (2g/kg)+Silymarin (100mg/kg)	6.290 \pm 0.109**
4	Paracetamol (2g/kg) + MESP (200mg/kg)	5.753 \pm 0.1201**
5	Paracetamol (2g/kg) + MESP (300mg/kg)	6.348 \pm 0.1076***

Values are expressed as mean \pm SD, n=6 statistical significance test was done by ONE-WAY ANOVA, followed by Dunnet's t-test. **P < 0.01. ***P<0.001

Table 2: Effect of methanolic extract of *Solanum pubescens* on liver enzymatic and non enzymatic antioxidants of paracetamol induced oxidative stress in Albino rats.

Parameters	Group I	Group II	Group III	Group IV	Group v
Glutathione	0.5367 \pm 0.03724	0.422 \pm 0.08446	0.5667 \pm 0.0682**	0.5733 \pm 0.8664**	0.5833 \pm 0.08017***
Lipid peroxide	0.4383 \pm 0.06494	0.5367 \pm 0.03141	0.4150 \pm 0.0680**	0.4167 \pm 0.06121**	0.4183 \pm 0.05636***
Glutathione peroxide	0.5650 \pm 0.04593	0.3567 \pm 0.0588	0.5433 \pm 0.03670**	0.56 \pm 0.05621**	0.5617 \pm 0.0534***
Super oxide dismutase	5.017 \pm 0.5636	3.500 \pm 0.2214	5.175 \pm 0.5037**	5.317 \pm 0.2368**	5.375 \pm 0.3060***
Catalase	51.50 \pm 2.405	26 \pm 5.756	45.83 \pm 2.482**	46.17 \pm 2.75**	48 \pm 2.113***
Na ⁺ /K ⁺ ATPase	0.0053 \pm 0.0005612	0.003817 \pm 0.0005154	0.00545 \pm 0.0005891**	0.005167 \pm 0.0005886**	0.005267 \pm 0.000588***

Values are expressed as mean \pm SD, n=6 statistical significance test was done by ONE-WAY ANOVA, followed by Dunnet's-test. **P < 0.01. ***P<0.001

DISCUSSION

The results of antioxidant property of methanolic extract of *Solanum pubescens* on paracetamol treated rats were shown on Table 1 and 2. The Total protein level increased in herbal plant treated group when compared to paracetamol treated group. The antioxidant enzymes GSH, LPO, SOD, Glutathione peroxidase, Catalase were significantly decreased and Lipid peroxidation was increased in paracetamol treated rats when compared to control group. The treatments with methanolic extract of *Solanum pubescens* Willd., significantly reverse the levels of GSH, SOD, CAT, Glutathione peroxidase, and Lipid peroxidation when compared to paracetamol group.

CONCLUSION

From the present study it is concluded that the methanolic extract of *Solanum pubescens* Willd., is a potent antioxidant and free radical scavenger.

REFERENCES

- V. Katalinic, M. Miols, T. K. Ulisic, M. Jukic, "Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols", *J. food chemistry*; 94; 2006: 550-557.
- Mohankumar Thenmozhi, Siva Sindhura, Krishnan Kannabiran, "Characterization of Antioxidant activity of *Streptomyces species VITTK3* isolated from Puducherry Coast, India". *Journal of Advanced Scientific Research*. 2010, 1(2); 46-52
- Omale James and B. Omajali Jacob, Evaluation of bio-safety and antioxidant activity of the fruit and leaf of *Saba florida (Benth.)* from Ibaji forest. *International Journal of Medicine and Medical Sciences* Vol. 2(3) pp. 100-105, March 2010.
- B.N. Ames, L.S. Gold, W.C. Willet (1995). "The causes and prevention of cancer". *Proc. Natl. Acad. Sci.* 92:5258-5265.
- K. Hemamalini and Vijusha M., "Antidiabetic activity of Methanolic extracts of Leaves of *Anogeissus acuminata Roxburgh ex candolle* and *Solanum pubescens Willd* by Alloxan induced model in Rats". *Der Pharmacia Letter*, 2012, 4 (5):1445-1460.
- K. Hemamalini, V. Ramya krishna, Dr. Anurag bhargav and Dr. Uma Vasireddy, "Hepatoprotective activity of *Tabebuia rosea* and *Solanum pubescens* against paracetamol induced hepatotoxicity in rats". *Asian J. Pharmaceuti. and Clin Res.* 2012; Vol 5: Issue 4.
- K. Hemamalini, P. Ashok, G. Sunny, S. Kumar Reddy, G. Ganesh, K. Santoshini, K. Rashmita, P. Priyanka, Y. Jayasri, T. Vishwanath, and Uma Vasireddy; "Gastroprotective Activity of *Gymnosporia emerginata*, *Solanum pubescence* and *Anogeissus acuminata* Leaf Extract against Ethanol-induced Gastric mucosal injury in Rats". *Inte. J. Pharmaceut. and Biomed. Res.*, 2011: 2(1), 38-42,
- P. Niyogi, N.J. Raju, P.G. Reddy, B.G. Rao. "Formulation and Evaluation of Anti-inflammatory activity of *Solanum pubescens* Willd extracts gel on albino Wister rats". *Inte. J. Pharmaceutics* 2012; 2(3): 484-490.
- J. Gandhiappan, R. Rengasamy. "Comparative evaluation of Antimicrobial activities of the members of *Solanaceae*". *Der Pharmacia Sinica*, 2012; 3 (3): 357-360.
- K. Hemamalini, Uma Vasireddy, G. Vamshi, K. Harinath, E. Vishnu Vardhan., A. Nagarjun Goud, H. Raghu and T. Sharath Goud, "In-vitro Anthelmintic Activity of *Solanum pubescens* and *Gymnosporia emerginata* Leave". *Inte. J. Pharma and Bio Sciences.* 2011; 2(3).
- K. Hemamalini K, Umavasi Reddy, M. Viswaja, Y. Nagarjuni, V. Sandhya rani and G.Vinitha "In-vitro Screening of Anti Lice Activity of Three different Leaves". *Int. J. Phytopharmacy Rese.* 2011 2(2): 54-57.
- G.N. Krishna kumari, L. Jagan Mohan Rao, N.S. Prakasa Rao. "Flavonol 3-O-Methyl Ethers From *Solanum pubescens*". *J. Nat.Prod.* 48(1), 149-150, 1985.
- C. Deepika Thenmozhi, V. Premalashmi, "Antioxidant Effect of Hydroethanolic Extract of *Hybanthus enneaspermus* on Paracetamol Induced Oxidative Stress in Albino Rats". *Int J. Res. in Pharmaceut. and Biomed. Sciences:* Jul – Sep 2011; Vol. 2 (3)
- L.N. Ebenyi, U. A. Ibiom and P. M. Ajar, "Effects of *Alliums sativum* extract on paracetamol – induced hepatotoxicity in albino rats". *Int. Res J. Biochem and Bioinf*, May 2012 ; Vol. 2(5): 93-97,
- O.H. Lowry, N. Rosebrough, J. Farr, R.J Randall, "Protein measurement with folin phenol reagents". *J. Bio. chem.* 1951; 193: 265-275.
- H. Ohkawa, N. Ohishi, N. Yagi, "Assay of LPO in animal tissues by TBA reactions". *Anal. Bio chem.* 1970; 95: 351-358.
- E. Beutler, B.M. Kelly, "The effect of sodium nitrate on red cell glutathione". *Experientia*. 1963; 18: 96-7.
- P. Kakar, B. Dose, P.N. Viswanthan, A.C Maehly, B. Chance, In: "Methods of Biochemical Analysis". Glick D, editors. New York: *Inter. Science.*, 1954 ; Vol. I: 357
- A.K. Sinha "Colorimetaaric assay of catalase". *Anal. Biochem.*, 1972; 47: 389-95.
- J.T. Rotruck, I.T Ganther, "Selenium: Biochemical role as a component of glutathione peroxidase purification and assay". *Curr Sci.*, 1973; 179: 588-590.