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ANTIOXIDANT EFFICACY OF *SESBANIA GRANDIFLORA* LINN IN ALLOXAN INDUCED WISTAR ALBINO RATS

Ruth Christiya.C[1], Dr. J. Radhika[*2] and Jothi. G[2]

[1]Research Scholar, Department of Biochemistry, Srimad Andavan Arts and Science College, (Autonomous), Tiruchirappalli- 620005.

[2]Department of Biochemistry, Srimad Andavan Arts and Science College, (Autonomous), Tiruchirappalli- 620005.

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***Correspondence for Author**
Dr. J. Radhika,
Department of Biochemistry, Srimad Andavan Arts Science College, (Autonomous) Tiruchirappalli-620005

ABSTRACT

Generation of free radicals during metabolism and other activities beyond the antioxidant capacity of a biological system results in oxidative stress, leading to cancer, heart disease, neurodegenerative disease etc. Plants have been an essential part of human life since the Vedic era. Recently there is an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical induced tissue damage. The present study was designed to analyse the antioxidant efficacy of *Sesbania grandiflora* Linn in alloxan induced albino rats. Animals were grouped into six and each group consist of six rats each. Group I- normal rats(Control) , Group II- Alloxan induced rats, Group III - Alloxan induced + plant extract(500 mg/kg b.wt) treated rats, Group IV- Alloxan induced + plant extract(750mg/kg b.wt) treated rats, Group V- Alloxan induced + glibenclamide treated rats and Group VI - plant extract (750 mg/kg b.wt) treated rats. Animals were screened for the parameters such as Total protein, LPO and antioxidant enzyme levels. Alloxan induced group showed a marked decrease in the level of total protein and antioxidant status and also a marked elevation of LPO. The plant extract administered orally to the alloxan induced rats for 45 days, produced significant decrease in the level of LPO and also produced marked increase in total protein level and antioxidant enzymes such as GR, GPx, SOD, and CAT which was comparable to glibenclamide treated rats. The above results clearly depicts that aqueous extract of *Sesbania grandiflora* Linn possesses significant antioxidant property.

KEYWORDS: Oxidative stress, antioxidant, Glutathione reductase, Glutathione peroxidase.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like hydrogen peroxide, superoxide hydroxyl and nitric oxide radicals, collectively called as reactive oxygen species (ROS). ROS exerts oxidative stress rendering each cell to face about 10,000 oxidative hits per second.[1] Oxidative stress results from an imbalance between radical-generating and radical scavenging systems that has increased free radical production or reduced activity of antioxidant defences or both.

In recent years growing evidence has been accumulated indicating the involvement of reactive oxygen species (ROS) in the pathogenesis of a number of diseases. Among the cellular and extracellular components, lipids, proteins, enzymes, DNA and RNA form the major targets of these reactive species, and the resulting damages are associated with degenerative ("Oxidative") diseases. Most living organisms possess efficient enzymatic and non-enzymatic defense systems against excess production of ROS. In addition to reduced glutathione (GSH), there are other defense mechanisms against free radicals, such as the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), whose activities contribute to eliminate superoxide, hydrogen peroxide and hydroxyl radicals. Different external factors (smoke, diet, alcohol, some drugs) and aging decreases the capacity of protecting systems, resulting in disturbance of the redox equilibrium. Therefore, antioxidants that scavenge ROS may be great value in preventing the onset and or the progression of oxidative diseases.[2]

There is currently immense interest in natural antioxidants and their role in human health and nutrition[3] Considerable amount of data have been generated on antioxidant properties of food plants around the globe[4] On the other hand, the medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability.[5] Several medicinal plants have also been widely used in the Indian traditional system of medicine for the treatment of number of diseases.[6] Some of these plants have shown potent antioxidant activity.[4, 7] So, to contribute further to the knowledge of Indian traditional plants, our present study is focussed on *Sesbania grandiflora* Linn to determine its antioxidant and free radical

scavenging properties. *Sesbania grandiflora* Linn is an important agroforestry species with various pharmacologically important components like alkaloids, flavanoids, tannins, triterpenes, gums, mucilage and anthraquinone, glycosides. Hence it has been used empirically as a traditional remedy in folk medicine to treat various diseases such as catarrh, dysentery, fever, headache, smallpox, sorethroat and stomatitis.[8]

MATERIALS AND METHODS

PLANT MATERIAL

Sesbania grandiflora Linn were freshly collected from in and around Trichy. The plant was identified with the help of Flora of Presidency of Madras and authenticated with the Voucher specimen deposited at Rapinat Herbarium, Department of Botany, St.Joseph's College, Trichy.

EXTRACTION

Sesbania grandiflora Linn leaves was shade dried at room temperature and powdered with an electrical blender to obtain coarse powder. 200 gm of the plant powder was mixed with 1200 ml of water and stirred continuously until it was reduced to one third and filtered. The filterate was evaporated to dryness. Paste form of the extract obtained was subjected to Pre-clinical screening.

EXPERIMENTAL ANIMALS

Studies were carried out using healthy adult wistar strain of albino rats of both sexes (150-200g), obtained from TamilNadu Veterinary Sciences University, Chennai. The animals were grouped and housed in Polypropylene cages with six animals per cage and maintained under standard laboratory conditions. The animals were fed with standard pellet and fresh water *ad libitum*. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee.

EXPERIMENTAL DESIGN

Animals were divided into six groups of six rats each.

Group I	:	Normal Control
Group II	:	Disease control received intraperitoneal injection of alloxan (150mg/kg b.wt) as a single dose
Group III	:	Alloxan induced rats treated with aqueous extract of *Sesbania grandiflora* Linn (500mg /kg b.wt) for 45 days

Group IV : Alloxan induced rats treated with aqueous extract of *Sesbania grandiflora* Linn (750mg/kg b.wt) for 45 days.

Group V : Alloxan induced rats treated with Glibenclamide (200mg/kg b.wt for 45 days

Group VI : Normal rats received aqueous extract of *Sesbania grandiflora* Linn (750mg/kg b.wt) for 45 days.

After the experimental period animals were sacrificed by cervical decaptitation. Blood was collected and serum was separated by centrifuging at 3000 rpm for 10 minutes. Liver were dissected out and washed in ice-cold saline. Liver tissues were homogenized in 0.1 M Phosphate buffer, and pH 7.4 used for studying various biochemical parameters such as Protein,[9] Glutathione Reductase(GR),[10] Glutathione peroxidise(GPx),[11] Superoxide Dismutase (SOD),[12] Catalase (CAT)[13] and Lipid peroxidase(LPO)[14] and Serum was used to analyse the serum protein levels.[9]

STATISTICAL ANALYSIS

All the results were expressed as mean ± S.E. The data obtained was statistically analyzed by one –way analysis of variance (ANOVA) and p values < 0.05 were considered statistically significant.

RESULTS

The levels of serum and tissue protein were reduced in Group II rats when compared to normal rats (Table 1). The animal treated with the plant extract (Group III& IV) for 45 days showed an increase in the protein level, which was comparable to the glibenclamide treated groups. The Group VI animals did not show any marked variation in the protein level.

Table 1: Estimation of Serum and Tissue Protein in experimental animals

GROUPS	I	II	III	IV	V	VI
Serum Protein (mg/dl)	5.9±0.07	2.38±0.05*	3.96±1.02	5.32±2.03**	4.11±1.05	6.33±0.2
Tissue Protein (mg/g)	3.26± 0.43	1.12±0.69*	1.99±0.62	2.35±0.77**	2.15±0.67	3.01±0.11

Values are ± SEM, n=6

*p<0.05 statistically significant when compared with normal control.

**p<0.05 statistically significant when compared with Alloxan treated group.

The activities of GR, GPx, SOD, CAT and LPO in alloxan induced rats were represented in Table 2. The levels of GR, GPx, SOD and CAT were significantly reduced and LPO were increased in alloxan induced rats. Treatment with *Sesbania grandiflora* Linn extract showed reversal of all these parameters which was comparable to the glibenclamide treated groups.

Table 2: Effect of Aqueous extract of *Sesbania grandiflora* Linn on the LPO and activities GR, GPx, SOD, and CAT on experimental models

GROUPS	I	II	III	IV	V	VI
Glutathione reductase (units/g of tissue)	43.63± 0.74	23.22±0.50*	31.98±0.66**	32.29± 0.89	43.22±0.53**	42.50±1.13
Glutathione peroxidise (units/g of tissue)	46.81± 0.97	26.10± 0.76*	31.3± 0.73	41.44± 1.20**	43.06± 1.18**	44.02± 1.28
SOD (mμ epinephrine oxidized/ml/min/ mg protein)	3.52± 0.11	1.21±0.14*	2.32±1.01	2.91± 0.16**	3.12± 0.37**	3.33±1.24
Catalase (units/g of tissue)	68.24± 0.68	46.21± 1.17*	52.34± 0.73	62.5± 0.29**	66.74± 0.28**	67.41± 0.31
LPO (nmol/mg of protein)	2.09± 1.09	6.77±1.14*	4.44±0.31	2.51±1.06**	2.17±0.92**	2.06±0.26

Values are ± SEM, n=6

*p<0.05 statistically significant when compared with normal control

**p<0.05 statistically significant when compared with Alloxan treated group.

DISCUSSION

Antioxidant level in the tissue is an important factor which decides the susceptibility of individual tissue to oxidative stress. Oxidative stress plays an important role in tissue damage associated with most of the diseases especially diabetes and its complications. Alloxan has the ability to generate excess reactive oxygen species such as H_2O_2, $O_2^{\cdot -}$ and HO[15] which is evident from the results obtained.

Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in cellular damage and is the most sensitive enzymatic index in tissue injury.[16] SOD has been reported as one of the most important enzyme in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT

decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals.[17] Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide.

GPx is an antioxidant enzyme involved in the detoxification of hydrogen and lipid peroxides[18] and two third of which is present in the cytosol and one third in the mitochondria of the liver. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. Increased amount of GSSG are transported out of cells to maintain the normal ratio[19] but when accumulated inside the cell, GSSG creates oxidative stress, and various cellular components become vulnerable to damage by reactive oxygen species mainly membrane lipids, protein and DNA. GSH / GSSG ratio is maintained by enzymatic activities of GR and GPx. GR converts GSSG to GSH in the presence of NADPH, while GPx acts as an antioxidant. The decrease in GR activity in alloxan induced animals may be responsible for the higher levels of GSSG. The present study indicates a reduction in the activity of GR, GPx in alloxan induced rats.[20]

The GR, GPx, SOD and CAT play an important role as protective enzymes against free radial formation in tissues. These are the enzymes that destroy the peroxides and play a significant role in providing antioxidant defence to an organism. GPx and CAT are involved in the elimination of H_2O_2. SOD acts to dismutase superoxide radical to H_2O_2 which is then acted upon by GPx. The functions of all these enzymes are interconnected and lowering of their activities results in the accumulation of peroxides and increase oxidative stress.[21] The treatment with *Sesbania grandiflora* Linn increased the activity of these enzymes and thus may help to avoid accumulation of free radicals.

Lipid peroxidation was induced by glucose through activation of lipooxygenase enzymes[22] Free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies such as ageing, wound healing, oxygen toxicity, liver disorders, inflammation etc.[23] The increased level of LPO in tissues may be due to the relatively high concentration of early peroxidizable fatty acids in the tissues.

Under condition of severe oxidative stress, free radical generation leads to protein modification. Proteins may be damaged directly by specific interactions of oxidant free radicals with particular susceptible amino acids.[24] This could be due to increased

peroxidation in alloxan induced rats. *Sesbania grandiflora* Linn extract caused a noticeable elevation in the plasma total protein as compared with their normal levels. The restoration of the protein levels may be due to the potential of the plant to induce the activity of the liver and the antioxidant systems.

CONCLUSION

The present study revealed that the plant extract exhibited satisfactory scavenging effect in all the radical scavenging assays and the use of this plant would exert several beneficial effects by virtue of its antioxidant activity. This study supports the contention that traditional medicines remain a valuable source in the potential discovery of natural product pharmaceuticals. Significant antioxidant efficacy showed by *Sesbania grandiflora* Linn provides a scientific validation for the traditional use of the plant.

REFERENCES

1. Mondal SK, Chakraborty G, Gupta M, Mazumder UK. *In-vitro* antioxidant activity of *Diospyros malabarica* Kostel bark. Indian Journal of Experimental Biology, 2006; 44: 39-44.
2. Karnan R, Subramani K. Antioxidant properties of certain south Indian medicinal plants. Journal of medicinal plants studies, 2015; 3(2): 58-60.
3. Aruoma OI. Nutrition and health aspects of free radicals and antioxidants. Food Chem Toxicol, 1994; 32: 671-683.
4. Kaur C, Kapoor HC. Antioxidant activity and phenolic content of some Asian vegetables. International Journal of Food Science and Technology, 2002; 37: 153-161.
5. Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F. Screening of antioxidant activity of three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. Journal of Ethnopharmacology, 2003; 84: 131-138.
6. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian medicinal plants. 3rd ed., New-Delhi, India; Council of Scientific and Industrial Research: 1992.
7. Aqil F, Ahmad I, Mehmood Z. Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants. Turk J Biol, 2006; 30: 177-183.
8. Malviya Reeta, Sharma Ravindra, Mishra Akhilesh, Rama Murthy A. Agastya (*Sesbania grandiflora* Linn) Ayurvedic Approach. Universal journal of Pharmacy, 2013; 2(04): 1-5.
9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin Phenol reagent. J Biol Chem, 1951; 193: 265-275

10. Carlberg B, Manervick B. Glutathione levels in rat brain. J Biol Chem, 1975; 250: 5475-5480.
11. Rotruck JT, Pope AL, Ganther H, Swanson, Hafeman DG, HoesiraSelenium WG. Biochemical role as a component of glutathione peroxidise. Science, 1973; 588-590.
12. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD. J Biol Chem, 1972; 247: 3170-3175.
13. Sinha A K. Colorimetric assay of catalase. Anal Biochemal, 1972; 47: 389-394.
14. Ohkawa H, Ohishi M, Yagi K. Assay for lipid peroxide in animal tissue by barbituric acid reaction. Anal Bio Chem, 1979; 95: 351-358.
15. Umamaheswari M , Chatterjee TK. Pharmacog res, 2009; 1(1): 25-34
16. Curtis JJ and Mortiz M. Serum enzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. Gastroenterol, 1972; 62: 84–92.
17. Chance B, Greenstein DS. The mechanism of catalase actions-steady state analysis. Arch Biochem Biophys, 1992; 37: 301–339.
18. Brigelius-Flohe R, Banning A, Schnurr K. Selenium-dependent enzymes in endothelial cell function. Antioxid Redox Signal, 2003; 5: 205-215
19. Eklow L, Thor H, Orrenius S. Formation and efflux of glutathione disulfide studied in isolated rat hepatocytes. FEBS Let, 1981; 127: 125-129.
20. Shajeela PS, Kalpanadevi V, Mohan VR. Potential antidiabetic, hypolipidaemic and antioxidant effects of Nymphaea pubescens extract in alloxan induced diabetic rats. Journal of Applied Pharmaceutical Science, 2012; 02(02): 83-88.
21. Kaleem M, Asif M, Ahmed QU, Bana B. Antidiabetic and antioxidant activity of Anona squamosa extract in streptozotocin induced diabetic rats. Singapore med j, 2006; 47(8): 127-134.
22. Kaimal S, Sujatha KS, George S. Hyperlipidemic and antioxidant effects of fruits of Musa AAA (ShenKadalai) in alloxan induced diabetic rats. Indian J exp Biol, 2010; 48: 165-173
23. Gupta V, Sharma M. Protective effect of *Cinnamon tejpata* on lipid peroxide formation in isolated rat liver homogenate. Curr res J Biol Sci, 2010; 2: 246-249.
24. Chitra V, Venkata KRCHH, Varma P, Krishna Raju MVR, Jeya PraKash K. Study of Antidiabetic and free radical scavenging activity of the Seed extract of Strychnos Nuxvomica. International journal of Pharmacy and Pharmaceutical science, 2010; 2(1): 106-110.