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Original Article

FREE RADICAL SCAVENGING ACTIVITY OF *LANTANA ACULEATA* ROOT EXTRACT IN HYPERLIPIDEMIC RATS

VINOTH KUMAR, K.,[1] DAWOOD SHARIEF, S.,[1]✉ RAJKUMAR, R.,[1] ILANGO, B.,[2] NAUSHEEN DAWOOD.,[3] CHAMUNDEESWARI, D.[4] AND SUKUMAR, E.[5]

[1]School of Environmental Sciences, Post Graduate and Research Department of Zoology, The New College, Chennai 600 014, [2]Department of Biotechnology, The New College, Chennai 600 014, [3]Post Graduate and Research Department of Zoology, JBAS College for Women, Chennai 600 018, [4]College of Pharmacy, Sri Ramachandra University, Porur, Chennai-600 116, [5]Department of Applied Sciences, Higher College of Technology, Muscat, Sultanate of Oman.
E-mail: sdawoodsharief@gmail.com

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Abstract: Lantana aculeata is a common weed that grows abundantly in many parts of India. The aerial part of the plant is reported to be toxic while the roots were found to be non- toxic when tested in albino rats. The alcoholic extract of the roots showed a significant hypolipidemic activity in normal rats. Hence the roots were studied for their free radical scavenging potential in hyperlipidemic animals by administering the alcoholic extract (LAR) in doses of 25, 50 and 100 mg/kg for 30 days. The levels of LPO, non-enzymatic antioxidant (TRG) and enzymatic antioxidants viz. SOD, CAT and GPx that showed changes in diseased condition were reverted back to near normal values by LAR extract treatment of plasma, liver and heart tissues. The presence of flavonoids besides oleanolic acid in large amounts might have caused the observed effect.

Key words – *Lantana aculeata*, Free radicals, Hyperlipidemia.

INTRODUCTION

Lantana aculeata Linn. (Family: Verbenaceae) is an indigenous weed that has mention in Ayurveda to treat vitiated body conditions [1]. The aerial portion of the plant is reported to be toxic to cattle [2]. In normal rats, the alcoholic extract of the roots (LAR extract) did not reveal any toxic effect up to a dose of 3200 mg/kg b.w. but showed significant hypolipidemic activity [3]. Hence LAR extract was tested for its free radical scavenging potential in hyperlipidemic rats in addition to phytochemical screening to have an idea on the types of chemical compounds present so that they could be correlated to the studied activity.

MATERIALS AND METHODS

Plant extract: Mature roots of *L. aculeata* were collected in the month of October - November from Puducherry and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai where a voucher specimen has been deposited.

The roots were chopped into small pieces, shade dried and coarsely powdered. About 1.0 kg of the coarse powder was exhaustively extracted with 90% ethanol by cold percolation method. After 72 hours, the solvent was decanted and distilled off over boiling water bath. Further concentrations were done under reduced pressure using rotary flash evaporator and finally dried in dessicator. The yield of the root extract (LAR extract) was found to be 0.32% (w/w).

Phytochemical screening: About 20 gm of LAR extract was chromatographed over silica gel (60 – 120 mesh) and elution with hexane:ethyl acetate (1:1) gave an impure solid that was purified by rechromatography to afford a triterpenoid in fairly good yield. This compound was found to be oleanolic

acid by spectroscopic data as well as comparison with an authentic sample.

Antioxidant activity: Adult male albino rats (150-200 g) of Wistar strain were obtained from Tamil Nadu University of Veterinary and Animal Sciences, Chennai and were maintained according to the guidelines of CPCSEA (Reg. No. 324) under the supervision of Animal Ethical Committee in accordance with the Indian National Law on Animal Care and Use. They were fed on commercial pelleted chow obtained from Poultry Research Station, Chennai and water was provided *ad libitum*. The permission of the Departmental Ethical Committee was obtained for the study and the experiments were conducted as per the principles prescribed for laboratory animal use.

The animals were divided into five groups of six rats each. Group I acted as control that received only the vehicle (hydrogenated groundnut oil, HGNO). Group II animals were given only cholesterol (500 mg/kg) together with HGNO. Groups III-V were administered LAR extract in doses of 25, 50 and 100 mg/kg, 30 minutes after giving cholesterol with HGNO. The experiment was continued for 30 days and on day 31, the animals were sacrificed by decapitation. The blood was collected and heart and liver were dissected out, washed with 1% ice-cold saline and homogenized with tris buffer.

The levels of lipid peroxide (LPO) and total reduced glutathione (TRG) were estimated in plasma. The aliquots of heart and liver tissue homogenates were studied for lipid peroxide [4], super oxide dismutase [5], glutathione peroxidase [6], total reduced glutathione [7] and catalase [8].

Statistical Analysis: Statistical evaluation of analytical data was done by Student's t-test using SPSS package. The values are expressed as the mean ± SD and values with $P<0.01$, $P<0.001$, $P<0.05$ were considered significant.

RESULTS

Table 1 shows details of the results obtained during experiment. It is evident that all the biomolecules (except TRG in plasma, liver and heart) showed increase in cholesterol treated group when compared with normal animals in group I. The values of these parameters returned towards near normal in groups III – V that were treated with 25, 50 and 100 mg/kg b.w. doses of LAR extract respectively.

DISCUSSION

Free radicals formation due to high levels of cholesterol results in oxidative abuse. All normal cells are protected from such damage by antioxidant system, which detoxify the reactive substances. They include non-enzymatic antioxidant namely reduced glutathione (GSH) and enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and scavenging agents such as the tocopherols and ascorbic acid [9].

The lipids present within the membrane of the cells in higher organisms contain a large number of polyunsaturated fatty acid side chains. Such fatty acids are prone to undergo a process known as lipid peroxidation (LPO), which involves generation of carbon radicals [10].

In the present study, elevated levels of LPO in plasma and the tissues of liver and heart of cholesterol-treated rats revealed a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system. The significant decline in the LPO concentrations of LAR extract administered rats unveils its antioxidant efficacy in a dose dependent manner. The decrease in the levels of LPO in plasma, heart and liver tissues were pronounced in 100 mg/kg b.w. dosage.

The non-enzymatic antioxidant GSH as a substrate of glutathione-S-transferase enables the liver to detoxify many foreign compounds [11]. An increase in GSH level in plasma, heart and liver tissues with LAR extract treated animals revealed better antioxidant effect in a dose dependent manner.

The decrease in GSH content of liver in hyperlipidemic rats may probably be due to its increased utilization by the hepatocytes in an attempt to counteract the increased formation of lipid peroxides [12]. The antioxidant enzymes, such as SOD, CAT and GPx constitute a mutually supportive team of defence against ROS [13]. Superoxide dismutase eliminates oxygen giving rise to H_2O_2, which can be destroyed either by catalase, glutathione peroxidase or glutathione-S-tranferase consisting the basic defensive system against oxygen toxicity [14].

Table 1: Effect of *Lantana aculeata* root extract (*LAR)* on LPO, non-enzymatic and enzymatic antioxidants in hyperlipidemic rats

Treatment (mg/kg)	Plasma		Heart					Liver				
	LPO (nmol/mg protein)	TRG (mg/dl)	LPO (nmol/mg protein)	TRG (mg/g tissue)	SOD (U^A/mg protein)	CAT (U^B/mg protein)	GPx (U^C/mg protein)	LPO(nmol/mg protein)	TRG (mg/g tissue)	SOD (U^A/mg protein)	CAT (U^B/mg protein)	GPx (U^C/mg protein)
Group I Vehicle	2.55 ± 0.36	9.20 ± 0.60	0.90 ± 0.35	1.02 ± 0.06	4.88 ± 0.39	212.01 ± 2.82	28.15 ± 1.79	2.82 ± 0.54	8.90 ± 0.79	4.50 ± 0.47	25.16 ± 0.83	13.69 ± 1.63
Group II Cholesterol (500)	3.72 ± 0.33^{a***}	6.68 ± 0.55^{a***}	1.88 ± 0.39^{a***}	0.36 ± 0.04^{a***}	6.26 ± 0.70^{a**}	231.95 ± 1.55^{a***}	35.93 ± 2.10^{a***}	3.53 ± 0.40^{a*}	7.23 ± 0.40^{a***}	5.81 ± 0.27^{a***}	36.52 ± 0.59^{a***}	18.72 ± 2.56^{a**}
Group III LAR (25) + Cholesterol	2.72 ± 0.33^{a***}	9.47 ± 0.49^{a***}	0.72 ± 0.26^{a***}	1.81 ± 0.94^{a***}	6.75 ± 0.46^{a*}	239.77 ± 2.42^{a*}	39.58 ± 3.11^{a*}	2.22 ± 0.57^{b***}	11.40 ± 0.84^{a***}	6.72 ± 0.69^{a*}	28.41 ± 0.72^{a***}	28.38 ± 2.27^{a***}
Group IV *LAR* (50) + Cholesterol	2.42 ± 0.52^{a***}	10.48 ± 0.86^{a***}	0.53 ± 0.10^{a***}	2.35 ± 0.31^{a***}	7.09 ± 0.48^{a**}	275.31 ± 3.49^{a***}	44.22 ± 2.82^{a***}	1.85 ± 0.65^{a***}	13.60 ± 0.86^{a***}	9.54 ± 0.58^{a***}	39.45 ± 1.27^{a**}	33.51 ± 2.38^{a***}
Group V *LAR* (100) + Cholesterol	1.78 ± 0.39^{a***}	12.65 ± 0.49^{a***}	0.47 ± 0.18^{a***}	2.42 ± 0.34^{a***}	7.78 ± 0.93^{a***}	286.38 ± 2.17^{a***}	48.73 ± 1.82^{a***}	1.68 ± 0.43^{a***}	16.43 ± 0.65^{a***}	20.47 ± 1.49^{a***}	42.42 ± 1.36^{a***}	37.09 ± 1.73^{a***}

LAR – Lantana aculeata root; LPO - Lipid Peroxidation; SOD – Superoxide Dismutase; CAT - Catalase; GPx – Glutathione Peroxidase; TRG – Total Reduced Glutathione. U^A - µmoles of H_2O_2 utilized / sec / mg protein; U^B - Amount of enzyme required to give 50% inhibition of NBT reduction; U^C - µmoles of GSH utilized / minute Values represent mean ± SEM of six animals. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; ^{NS}-Non-significant; ^{a} - when compared to vehicle; ^{b} - when compared to cholesterol.

The major components of LAR extract are triterpenoids besides flavonoids and phenolic compounds. The latter scavenge free radicals, including hydroxyl and superoxide anions, inhibit lipid peroxidation and improve lipid profiles [15-18]. Both these components are also known to stimulate CAT and SOD gene transcription [19,20]. Catalase, a flavin linked oxidase, efficiently detoxifies hydrogen peroxide when it exceeds normal levels [21]. The increase in the level of CAT and SOD with LAR extract treated animals in different doses indicated the possible role of phenols and flavonoids in

modulating the expression of both CAT and SOD activities. The significant increase in CAT and SOD levels is comparable with the observation of Nishant and Narasimhacharya, who reported the role of flavonoids and polyphenols in increasing the above parameters [22].

Glutathione peroxidase appears to play an important role in the protection of cells from damage promoted by intracellular process [6]. LAR extract administration increased the glutathione peroxidase activity thus proving its scavenging ability against toxic radicals.

The ability of LAR extract to significantly increase the levels of the antioxidant enzyme systems with a concomitant decrease in the lipid peroxide levels and increase in GSH levels in the plasma and decrease in LPO levels with increase in GSH, SOD, CAT and GPx in the tissues of heart and liver is indicative of its protective role and its free radical scavenging ability in hypercholesterolemic condition. This abnormal condition has lead to renewed initiative to search for effective phytochemical compounds present in the extract.

Oleanolic acid is the major component present in the roots of *Lantana aculeata* [3]. It is a pentacyclic triterpenoid with a wide range of pharmacological properties [23] and also possesses significant antioxidant properties [24]. Perhaps oleanolic acid may be one of the active principals contributing to antioxidant actions of LAR extract. Further studies on the subject are in progress in our laboratory.

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REFERENCES

[1] Grover, J.K., Yadav, S. and Vats, V.: J. Ethnopharmacol., 81: 81-100 (2002).
[2] Seawright, A.A. and Allen, J.G.: Aust. Vet. J., 48: 323-331 (1972).
[3] Vinoth Kumar, K., *Studies on the Toxicity and Biological Significance of an Indigenous Weed Lantana Aculeata* Linn. PhD Thesis, University of Madras, India, pp. 63-64 (2008).
[4] Ohokawa, H., Ohisi, N. and Yagi, K.: Anal. Biochem., 95: 351-358 (1979).
[5] Marklund, S. and Marklund.: Euro. J. Biochem., 47: 469- 474 (1974).
[6] Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G.: Science, 179: 588-590 (1973).
[7] Moron, M.S., Despierra, J.W. and Mannervik, B.: Biochem Biophys. Acta, 585: 67- 78 (1979).
[8] Sinha, A.K.: Anal. Biochem., 47: 389-394 (1972).
[9] Hoffman, C.E.J., Webster, N.R., Wiggins, P.A., Chisholm, E.M., Giles, G.R. and Leveson, S.H.: Br. J. Cancer, 51: 127- 129 (1985).
[10] Sohal, R.S.. Age Pigments. In: *Metabolic Rate, Ageing and Lipofucin accumulation*, (Sohal, R.S. ed) Elsevier North Holland Biomedical Press, Amsterdam. pp. 303-311 (1981).
[11] Hazelton, G.A. and Lang, C.A.: Biochem. J., 88: 25-30 (1980).
[12] Shyamala, M.P., Venukumar, M.R. and Latha, M.S.: Ind. J. Pharmacol., 35: 99-103 (2003).
[13] Bandyopadhyay, U., Das, D. and Banerjee, R.K.: Curr. Sci., 77: 658-665 (1999).
[14] Gil, P., Alonso Bedate, M. and Barja De Quiroga, G.: Free Rad. Biol. Med., 3: 137-146 (1987).
[15] Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M. and Pridham, J.B.: Free Rad. Res., 22: 375-383 (1995).
[16] Tripathi, Y.B., Singh, B.K., Pandey, R.S. and Kumar, M.: Evid. Based Comp. Alternat. Med., 2: 217-221 (2005).
[17] Ljubuncic, P., Dakwar, S., Portnaya, I., Cogan, U., Azaizeh, H. and Bomzon, A.: Evid. Based Compl. Alternat. Med., 3: 329-338 (2006).
[18] Punitha, I.S.R., Rajendran, K., Shirwaikar, A. and Shirwaikar, A.: Evid. Based Compl. Alternat. Med., 2: 375-381 (2005).
[19] Toyokuni, S., Tanaka, T., Kawaguchi, W., Fang, N.R., Ozeki, M. and Akatsuka, S.: Free Rad. Res., 37: 1215-1224 (2003).
[20] Ranaivo, H.R., Rakotoarison, O., Tesse, A., Schott, C., Randriantsoa, A. and Lobstein, A.: Amer. J. Physiol. Heart Circ. Physiol., 286: 775-781 (2004).
[21] Ji, L.L., Stratman, F.W. and Lardy, H.A.: Arch. Biochem. Biophys., 263: 150-160 (1998).
[22] Nishant, P.V. and Narasimhacharya, A.V.R.L.: Evid. Based Compl. Alternat. Med., 1-8 (2007).
[23] Ravokatra, A., Nigeon-Dureuil, M. and Ratsimamaga, A.R.: CR Acad. Sci. Herbd. Seances. Acad. Sci. D., 278: 1743–1746 (1974).
[24] Zhang, L.Z. and Li, X.F.: Trad. Med. Clin. Pharm., 8: 24 - 26 (1992).