Antioxidant Activities of Hydroalcoholic Extract of *Ocimum sanctum* Against Cadmium Induced Toxicity in Rats

B. Ramesh · V. N. Satakopan

Received: 10 January 2009 / Accepted: 13 August 2009 / Published online: 25 August 2010
© Association of Clinical Biochemists of India 2010

Abstract The present study was undertaken to analyze the antioxidant (both enzymic and nonenzymic) activities of leaves of *Ocimum sanctum* hydroalcoholic extract against cadmium induced damage in albino rats. Oral administration of cadmium as CdCl_2 (6.0 mg/kg body weight) led to significant elevation of lipid peroxidation (LPO) levels and significantly decreased Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Reduced Glutathione (GSH) and Vitamin C (Ascorbate) levels. Administration of *Ocimum sanctum* extract (100 mg/kg body weight, po) and (200 mg/kg body weight, po) before and after cadmium intoxication showed a significant decrease in LPO levels and significant increase in SOD, CAT, GPx, GSH and Ascorbate levels. The results suggest that oral administration of *Ocimum sanctum* extract provides significant protection against cadmium induced toxicity in Wistar albino rats.

Keywords *Ocimum sanctum* · Cadmium chloride · Free radicals · Liver damage · Antioxidants

Introduction

Cadmium (Cd) is a very toxic heavy metal and an important environmental pollutant, which is present in the soil, water, air, food and in cigarette smoke. Cadmium causes poisoning in various tissues of humans and animals [1, 2]. The uptake of cadmium into the liver is critical for the development of overall toxicity induced by the heavy metal. Approximately

half of cadmium absorbed systemically is rapidly accumulated in the liver, which results in the reduced availability of cadmium to such organs as the kidneys and testes, which are more sensitive to its toxic actions [3]. The toxic effects of cadmium are due to its inhibition of liver metabolic enzyme systems containing sulphhydryl groups and uncoupling of oxidative phosphorylation in mitochondria [4], which results in increased lipid peroxidation, hepatic congestion, ischemia and hypoxia [5]. Production of ROS and oxidative tissue damage due to cadmium have been associated with hepatotoxicity [6]. Moreover, a variety of accompanying changes in antioxidant defense enzymes are reported [7]. It has been shown that free radical scavengers and antioxidants are useful in protecting against cadmium toxicity [8]. Great efforts have been made in an attempt to find safe and potent natural antioxidants from plant sources [9]. *Ocimum sanctum* Linn. (Labiatae), commonly known as holy basil, is an herbaceous plant found throughout the South Asian region [10]. The oil of *Ocimum sanctum* possesses antibacterial, antifungal, antioxidant and radioprotective properties [11]. Ancient Hindu literature is abundant with the medical actions of *Ocimum sanctum* [12]. Aqueous suspension of finely powdered *Ocimum sanctum* leaves were tested for their effect on intestinal transit in albino rats [13]. Holy basil has been shown also to be effective as antistress, adaptogenic and attenuates the stress-induced changes [14–16]. More studies revealed that *Ocimum sanctum* decreased lipid peroxidation and increased the activity of SOD [17]. Eugenol and ursolic acid from *Ocimum sanctum* have been reported to induce protection against free radical induced cellular damage [18, 19]. Keeping in view the pharmacological properties of *Ocimum*, present investigation has been undertaken to assess the antioxidant effect of *Ocimum sanctum* extract on cadmium induced oxidative stress in albino rats.

B. Ramesh (✉) · V. N. Satakopan
Department of Biochemistry, PSG College of Arts and Science,
Civil Aerodrome Post, Coimbatore 641 014, India
e-mail: ramesh2575@yahoo.com

Materials and Methods

Plant Material and Extraction

Ocimum sanctum was collected from the local areas of Coimbatore, identified by a taxonomist and a voucher specimen (No. BSI/SC/5/23/08-09/Tech-1535) was deposited at Botanical Survey of India, Southern circle, Tamilnadu Agricultural University, Coimbatore, India. Fresh leaves of *Ocimum sanctum* were dried under shade and powdered with a mechanical grinder to obtain a coarse powder. 1 kg of powdered material was then subjected to cold maceration with 50% alcohol (1.5 l ethanol and 1.5 l water) for 3 days with intermittent shaking, filtered, evaporated and vacuum dried. A brownish residue weighing 15.5% (w/w) was obtained and kept in air tight bottles until use. The chemical constituents of the extract were identified by qualitative analysis [20].

Animals and Experimental Design

Male albino rats (Wistar strain) weighing 150–200 g were obtained from animal breeding center, PSG Institute of Medical Sciences & Research, Coimbatore, Tamilnadu, India. They were housed in PSG College of Arts & Science, Coimbatore, Tamilnadu, India, in controlled temperature ($27 \pm 2^\circ\text{C}$), humidity ($55 \pm 10\%$) and light with 12:12 h L:D cycle. Animals were fed with standard pellet (Hindustan Lever Ltd., India). They were given a week time to get acclimatized with laboratory condition. Ethical clearance for the handling of experimental animals was obtained from the committee constituted for the purpose (158/1999/CPCSEA).

After acclimatization the rats were divided into six main groups (six rats in each group).

Group I: Normal control

Group II: Cadmium (6 mg/kg body weight/day) as CdCl_2 orally for a period of 30 days [21]

Group III: Cadmium as in group II + *Ocimum sanctum* extract (100 mg/kg body weight, po) 10 consecutive days before CdCl_2 administration and until 30 days of CdCl_2 administration [22]

Group IV: Cadmium as in group II + *Ocimum sanctum* extract (200 mg/kg body weight, po) 10 consecutive days before CdCl_2 administration and until 30 days of CdCl_2 administration

Group V: *Ocimum sanctum* extract (100 mg/kg body weight) and

Group VI: *Ocimum sanctum* extract (200 mg/kg body weight)

At the end of the experimental period, the rats deprived of food overnight and sacrificed by light ether anesthesia.

Liver was removed and cleaned in normal saline. A known weight of liver was then homogenized (10% w/v) in ice cold phosphate buffer (0.1 M, pH 7.4) using potter–Elvehjem Teflon homogenizer. The homogenate was centrifuged at 5000 rpm at 4°C for 30 min and supernatant obtained was used for the assay of various enzymes.

Biochemical Estimations

To find out the antioxidant effect of the sample, the following parameters were analyzed. LPO [23], SOD [24], CAT [25], GPx [26] GSH [27] and Ascorbate [28].

Statistical Analysis

The results were presented as the mean $\pm$ SEM. One-way ANOVA, followed by Duncan's Multiple Range Test was adopted to all the parameters under study to test the level of statistical significance.

Results and Discussions

Phytochemical Studies

Table 1 shows the phytochemical constituents present in the *Ocimum sanctum* hydroalcoholic extract.

It has been reported that the flavonoid constituents of the plant possess antioxidant properties [29]. Phenolics are highly effective free radical scavengers and exhibit strong antioxidant activity [30]. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential [31].

Studies on Antioxidant Enzymes

Table 2 indicates the levels of enzymic and non-enzymic antioxidants in the liver of various groups.

Group II rats showed a significant ($P < 0.01$) increase in the liver LPO levels when compared to Group I rats. Lipid peroxidation has been postulated to the destructive process of liver injury due to cadmium administration. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals [32]. Group III and Group IV rats administered with low and high doses of *Ocimum sanctum* extract showed a significant ($P < 0.01$) decrease in the liver LPO levels when compared to Group II rats. This may be due to the presence of flavonoids and phenolic compounds which have been recognized as excellent

Table 1 Phytochemical constituents present in the *Ocimum sanctum* hydroalcoholic extract

Alkaloids	Flavonoids	Saponins	Phenols	Tannins	Glycosides	Proteins	Carbohydrates
+	+	+	++	+	++	+	+

Table 2 Levels of enzymic and non-enzymic antioxidants in the liver of various groups

Groups	LPO ^a	SOD ^b	CAT ^c	GPx ^d	GSH ^e	Vitamin C ^f
I	0.80 ± 0.08	12.35 ± 2.23	68.46 ± 8.48	6.47 ± 0.94	1.93 ± 0.30	4.01 ± 0.39
II	2.47 ± 0.15*	6.07 ± 2.81*	34.01 ± 8.82*	4.40 ± 0.27*	0.73 ± 0.28*	2.28 ± 0.23*
III	1.40 ± 0.07*	8.85 ± 0.22*	49.12 ± 6.14*	5.94 ± 1.83**	1.47 ± 0.28*	2.88 ± 0.21*
IV	1.32 ± 0.15*	9.63 ± 2.21*	59.36 ± 8.85*	6.02 ± 1.78**	1.65 ± 0.35*	3.11 ± 0.26*
V	0.97 ± 0.09	12.19 ± 2.89	63.80 ± 8.66	6.39 ± 1.05	1.93 ± 0.30	3.82 ± 0.22
VI	0.75 ± 0.08	12.27 ± 2.10	68.41 ± 6.92	6.29 ± 1.00	2.02 ± 0.45	3.82 ± 0.23
	LSD(1%)0.17	LSD(1%)3.58	LSD(1%)12.77	LSD(5%)1.49 LSD(1%)2.00	LSD(1%)0.52	LSD(1%)0.42

Values are mean ± SD (n = 6)

Statistical significance are as follows: * $P < 0.01$, ** $P < 0.05$

Comparisons between groups are as follows

Group I versus Group II; Group II versus Group III and Group IV; Group I versus Group V and Group VI; ^a μmoles/g tissue; ^b U/mg protein (amount of enzyme required to inhibit 50% reduction of NBT); ^c U/mg protein (μm of H₂O₂ decomposed/min/mg protein); ^d U/mg protein (μ moles of GSH consumed/min/mg protein); ^e μg/mg protein; ^f μg/mg protein

scavengers of superoxide, hydroxyl ion and peroxy radicals thereby inhibiting lipid peroxidation [33].

In Group II rats there was a significant ($P < 0.01$) decrease in SOD, CAT, GPx, GSH and Ascorbate levels in the liver when compared to Group I rats. The decrease in SOD levels may be due to inactivation of SOD by either cadmium induced lipid peroxidation [34] or the antagonistic effect of cadmium with copper and zinc, which are important metals for the activity of SOD molecule [35]. The reduction in the activity of Catalase is due to the accumulation of superoxide radicals and hydrogen peroxide.

The decrease in GPx levels could be due to insufficient disposal of peroxides and results in elevated lipid peroxidation [36]. The decrease in GSH levels in the liver when compared to Group I rats could be probably due to either increased utilization of GSH by the cells to act as scavengers of free radicals caused by toxic chemical agents, or enhanced utilization of GSH by GPx [37]. The decrease in Vitamin C levels in the liver may be due to an increased reaction of Vitamin with ROS in the defense process. Vitamin C exist in the interconvertible reduced and oxidized forms [38] thus participate in neutralizing free radicals as and when they are formed.

In the present study, Group III and Group IV rats showed a significant ($P < 0.01$) increase in the levels of SOD, CAT, GSH, Ascorbate levels and significant ($P < 0.05$) increase in the GPx levels when compared to

Group II rats. This shows that the *Ocimum sanctum* extract can reduce reactive free radicals that might lessen oxidative damage to the liver and improve the activities of the hepatic antioxidant enzymes like SOD and CAT, protecting the liver from cadmium intoxication. The increase in GPx levels in the liver may be due to increased supply of GSH for the activation of GPx [26]. *Ocimum sanctum* probably increased the levels of reduced glutathione by facilitating reduction of oxidative free radicals by H donation [39]. The increase in ascorbate levels may be due to a defense response of the organism to oxidant injuries caused by cadmium [40], indicating that the extract has the ability to combat produced free radicals, enhancing the recovery of the animals from cadmium induced damage, compared to the untreated group. No significant difference was found in all the parameters in the liver of Group V and Group VI rats when compared to Group I rats.

Conclusion

This dose dependent suppression of cadmium induced adverse effects on liver antioxidants status suggest that the hydroalcoholic extract of *Ocimum sanctum* has antioxidant activity based on free radical scavenging or modulation of antioxidant status and tissue regeneration in the liver of cadmium intoxicated rats.

Acknowledgments The authors are thankful to the management of PSG College of Arts & Science for providing laboratory facilities to carryout this research work.

References

- Swiergosz R, Zaurzewska M, Sawicka-Kapusta K, Bacia K, Jankowska I. Accumulation of cadmium and its effect on bank vole tissues after chronic exposure. *Ecotoxicol Environ Saf*. 1998;41:130–6.
- Stohs SJ, Bagachi D, Hassoun E, Baguchi M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. *J Environ Pathol Toxicol Oncol*. 2000;19:201–13.
- Del Raso NJ, Foy BD, Gerhart JM, Frazier JM. Cadmium uptake kinetics in rat hepatocytes correction for albumin binding. *Toxicol Sci*. 2003;72:19–30.
- Williams F, Robertson R, Roworth M. Detailed profile of 25 major organic and inorganic substances. Glasgow: SCEIH; 1999. pp. 12–19.
- Habeebu SS, Liu J, Klaassen CD. Cadmium induced apoptosis in mouse liver. *Toxicol Appl Pharmacol*. 1998;149(2):203–9.
- Chin TA, Templeton DM. Protective elevations of glutathione and metallothionein in cadmium-exposed mesangial cells. *Toxicology*. 1993;77:145–56.
- Zikic RV, Stajn A, Ognjanovic B, Saicic ZS, Kostic MM, Pavlovic SZ, Petrovic VM. The effect of cadmium and selenium on the antioxidant enzyme activities in rat heart. *J Environ Pathol Toxicol Oncol*. 1998;17:259–64.
- Fariss MW. Cadmium toxicity: unique cytoprotective properties of alpha-tocopheryl succinate in hepatocytes. *Toxicology*. 1991;69:63–77.
- Lee SE, Ju EM, Kim JH. Free radical scavenging and antioxidant enzyme fortifying activities of extracts from *Smilax china* root. *Exp Mol Med*. 2001;33(4):263–8.
- Hannan JMA, Marenah L, Ali L, Rokeya B, Flatt PR, Abdelwahab YHA. *Ocimum sanctum* leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic β -cells. *J Endocrinol*. 2006;189:127–36.
- Sharma MK, Kumar M, Kumar A. *Ocimum sanctum* aqueous leaf extract provides protection against mercury induced toxicity in Swiss albino mice. *Ind J Exp Biol*. 2002;40:1079–82.
- Ubaid FS, Anantrao KM, Jaju JB, Mateenuddin MD. Effect of *Ocimum sanctum* leaf extract on hepatotoxicity induced by antitubercular drugs in rats. *Ind J Phy Pharm*. 2002;47(4):465–70.
- Singh V, Singh A, Nath R, Mishra N, Dixit KS, Singh N. Effect of some anti-stress plant drugs on the intestinal transit. *J Biol Chem Res*. 1991;10(4):601–2.
- Bhargava KP, Singh N. Anti-stress activity of *O. sanctum*. *Ind J Med Res*. 1981;73:443–51.
- Singh N, Mishra N, Srivastava AK. Effect of antistress plants on biochemical changes during stress reactions. *Ind J Pharmacol*. 1991;23:137–42.
- Singh N, Verma P, Mishra N. A Comparative evaluation of some antistress agents of plant origin. *Ind J Pharmacol*. 1991;23:99–103.
- Panda S, Kar A. *Ocimum sanctum* leaf extract in the regulation of thyroid function in the lame mouse. *Pharmacol Res*. 1998;38(2):107–10.
- Balanehr S, Nagarajan B. Intervention of adriamycin induced free radical damage by ursolic acid. *Biochem Int*. 1992;28:735.
- Rajkumar DV, Rao MNA. Dehydrozingerone and isoeugenol as inhibitors of lipid peroxidation and as free radical scavengers. *Biochem Pharmacol*. 1993;46:2067.
- Kokate CK. Preliminary phytochemical screening. In: *Practical pharmacognosy*, IV ed. New Delhi: Vallabh Prakashan; 2002. p. 107–11.
- Jeyaprakash K, Chinnaswamy P. Effect of Spirulina and Liv 52 on cadmium induced toxicity in Albino rats. *Ind J Exp Biol*. 2005;43:773–81.
- Kath RK, Gupta RK. Antioxidant activity of Hydroalcoholic leaf extract of *Ocimum sanctum* in animal models of peptic ulcer. *Indian J Physiol Pharmacol*. 2006;50(4):391–6.
- Niehius WG, Samuelsson D. Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. *Eur J Biochem*. 1968;6:126–30.
- Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys*. 1984;21:130–2.
- Sinha AK. Colorimetric assay of catalase. *Anal Biochem*. 1972;47:389–94.
- Rotruck JT, Pope AL, Genthner H, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science*. 1973;179:588–90.
- Moron MJ, Depierre JW, Mannirvik B. Levels of GSH, GR and GST activities in rat lungs and liver. *Biochem Biophys Acta*. 1979;582:67.
- Roe JH, Kuether CA. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenyl hydrazine derivative of dehydro ascorbic acid. *J Biol Chem*. 1943;147:399–407.
- El-Seedi HR, Nishiyama S. Chemistry of bioflavonoids. *Ind J Pharm Educ*. 2002;36:191–4.
- Hsu C-Y. Antioxidant activity of extract from *Polygonum aviculare* L. *Biol Res*. 2006;39:281–8.
- Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med*. 1996;20:933–56.
- Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam D, Sengupta P, Maity BC, Maity TK. Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus frutescens* (Linn) R.Br. on paracetamol-induced hepatotoxicity in rats. *Trop J Pharm Res*. 2007;6(3):755–65.
- Bem EM, Piotrowski JK, Sobczak-Kozłowska M, Dmuchowski C. Cadmium, zinc, copper and metallothionein levels in human liver. *Int Arch Occup Environ Health*. 1988;60(6):413.
- Tang W, Sadovic S, Saikh ZA. Nephrotoxicity of cadmium-metallothionein protection by zinc and role of Glutathione. *Toxicol Appl Pharmacol*. 1988;151:276.
- Gasiewicz TA, Smith JC. Interaction of cadmium and selenium in rat plasma in vivo and in vitro. *Biochem Biophys Acta*. 1978;428:113.
- Suja V, Sharmila SL, Shyamala Devi C. Protective effect of Liv.52 and Liv.100 ayurvedic formulation on lipid peroxidation in rat liver homogenate as in vitro study. *Ind J Exp Biol*. 1997;35(1):50.
- Larson RA. The antioxidants of higher plants. *Phytochemistry*. 1988;27:969.
- Lakshmi Devi S, Kannappan S, Anuradha CV. Evaluation of invitro antioxidant activity of Indian bay leaf, *Cinnamomum tamala* (Buch-Ham.) T.Nees and Ebrum using rat brain synaptosomes as model system. *Ind J Exp Biol*. 2007;45:778–84.
- Sethi J, Sood S, Seth S, Talwar A. Evaluation of hypoglycemic and antioxidant effect of *Ocimum sanctum*. *Ind J Clin Biochem*. 2004;19(2):152–5.
- Ognjanovic BI, Pavlovic SZ, Maleti SD, Zikic AS, Stajn ZS, Radojicic RM, Saicic ZS, Petrovic VM. Protective influence of Vitamin E on antioxidant defense system in the blood of rats treated with cadmium. *Physiol Res*. 2003;52:563–70.