Antidyslipidemic and Antioxidant Activities of *Hibiscus rosa sinensis* Root Extract in Alloxan Induced Diabetic Rats

Vishnu Kumar · Farzana Mahdi · Ashok Kumar Khanna ·
Ranjana Singh · Ramesh Chander · Jitendra Kumar Saxena ·
Abbas Ali Mahdi · Raj Kumar Singh

Received: 30 November 2011 / Accepted: 2 May 2012 / Published online: 4 June 2012
© Association of Clinical Biochemists of India 2012

Abstract The antidyslipidemic activity of *Hibiscus rosa sinensis* (Malvaceae) root extract has been studied in alloxan induced diabetic rats. In this model, oral administration of root extract (500 mg/kg bw. p.o.) for 15 days resulted in significant decreased in the levels of blood glucose, plasma lipids and reactivated post heparin lipoprotein lipase activity in alloxan induced diabetic rats. Furthermore, the root extract (50–500 µg) when tested for its antioxidant activity, inhibited the generation of super oxide anions and hydroxyl radicals, in both enzymic and non enzymic systems in vitro. The results of the present study demonstrated antidyslipidemic and antioxidant activities in root extract of *H. rosa sinensis* which could be used in prevention of diabetic-dyslipidemia and related complications.

Keywords *Hibiscus rosa sinensis* ·
Anti-dyslipidemic agent · Natural antioxidants ·

Post heparin lipolytic activity · Alloxan induced-diabetes ·
Hypoglycemic agent

Introduction

Hibiscus rosa sinensis Linn (known as Gudhal in Hindi, Japa in Sanskrit, and Shoe Flower in English) is mentioned in ancient medical literature to possess anti-tumor, detoxifier, antifertility, and wound healing activities [1]. Recent researches have found a variety of pharmacological effects of almost all the parts of this plant [2].

Diabetes mellitus with an alarmingly rising incidence, is a cluster of abnormal metabolic paradigm having a common feature of hyperglycemia and dyslipidemia, has been a leading cause of death in both industrialized and developing nations. Apart from hyperglycemia, disorders of lipid metabolism following oxidative stress are the prime risk factors for initiation and progression of diabetic complications in patients and thus there is an urgent need for a simultaneous treatment [3]. The known lipid lowering drugs such as fibrates, statins, bile acid sequestrants and antidiabetic drugs such as glibenclamide, metformin are not so effective against diabetic-dyslipidemia and also cause many side effects in patients [4]. Therefore, research and development of a lipid lowering drug with antidiabetic and antioxidant potential altogether, from natural products are the best option and also are in great demand. We had earlier reported lipid lowering effect of ethanol extract of root of this plant in two different models of hyperlipidemia in rats [5]. In continuation the present study was designed to investigate possible antidyslipidemic and antioxidant activity of the root in alloxan induced diabetic rats.

V. Kumar · F. Mahdi (✉) · R. Chander
Department of Biochemistry, Era's Lucknow Medical College &
Hospital, Sarfarazganj, Hardoi Road, Lucknow 226003, India
e-mail: farzana.mahdi@gmail.com

A. K. Khanna · J. K. Saxena
Division of Biochemistry, Central Drug Research Institute,
Lucknow, India

R. Singh · A. A. Mahdi
Department of Biochemistry, C.S.M. Medical University,
Lucknow, India

R. K. Singh
Department of Biochemistry, Shri Guru Ram Rai Institute of
Medical & Health Sciences, Dehradun, India

Materials and Methods

Preparation of Root Extract

Preparation of root extract was done as described in our earlier paper [5]. The extract was stored in refrigerator and used for in vivo and in vitro experiments. The root extract was not soluble in water. One gram of dried mass was crushed and a paste was prepared in distilled water containing 2 % w/v gum acacia. The paste was diluted with continuous triturating with above vehicle to a final volume of 20 ml. This homogenous suspension contained 50 mg drug/ml. A dose of 1 ml/100 g bw of rat i.e. 500 mg/kg bw was fed orally once daily for 15 days by feeding canula. Similarly glibenclamide suspension (60 µg/ml) was also prepared and administered as above. The freshly prepared drugs were used every day.

Antidyslipidemic activity in Alloxan induced diabetic rats. The rats were maintained exactly as in our previous study [5]. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate 150 mg/kg bw. After three days of injection, diabetes was confirmed by glucometer. The rats with serum glucose level 280–320 mg/dl were taken for the study.

Experimental Design

The rats were divided in four groups having six animals in each as follows: Group 1: control rats (on normal saline); Group 2, Alloxan treated diabetic rats (on normal saline); Group 3, Alloxan treated diabetic rats + *H. rosa sinensis* (500 mg/kg b.w); Group 4, Alloxan treated diabetic rats + glibenclamide (600 µg/kg b.w). After 15 days of feeding rats were fasted overnight, anaesthetized with thiopental solution, and injected (ip) with 1 ml/kg bw of 10 mg/ml solution of heparin. After 15 min blood was withdrawn from the retro-orbital plexus and collected in EDTA coated tubes. The blood was used for the estimation of glucose, simultaneously plasma was separated and used for the estimations of total cholesterol: TC, phospholipids: PL, triglyceride: TG, free fatty acids: FFA, plasma posts heparin lipolytic activity (PHLA) and lipid peroxide levels by standard spectrophotometric methods mentioned in our earlier paper [5].

In Vitro Anti-oxidant Activity

Enzymic and Non Enzymic Generation of Superoxide Anions

The effect of *H. rosa sinensis* root extract on the generation of superoxide anions ($O_2^{\cdot-}$) in vitro, in an enzymic system of xanthine–xanthine oxidase was investigated [6].

Xanthine oxidase activity in system (A) containing xanthine and different concentrations of root extract (50–500 µg) added with 0.03 U/ml of xanthine oxidase in phosphate buffer, was assayed spectrophotometrically at 295 nm. The change in optical density corresponding to amount of uric acid formed was compared with reaction mixture which did not include with their test substance. The influence of root extract on nitro blue tetrazolium (NBT) reduction by $O_2^{\cdot-}$ anions, was measured in a reaction mixture (B) containing xanthine oxidase and NBT in absence or presence of extract (50–500 µg). After incubation, the amount of formazone formed was measured at 560 nm on spectrophotometer. Another system employed for non enzymic generation of $O_2^{\cdot-}$ anions was comprised of phenazine methosulphate, NADH and NBT [7]. After 90 s incubation in absence or presence of test extract 50–500 µg, the amount of formazone formed was read at 560 nm against respective reagent blank.

Enzymic and Non Enzymic Generation of Hydroxyl Radical

Hibiscus rosa sinensis root extract (50–500 µg) was tested against the formation of hydroxyl radicals ($OH^{\cdot}$) in vitro in an enzymic system composed of sodium salicylate, $FeSO_4$, hypoxanthine and xanthine oxidase, assayed for 2,3-dihydroxybenzoate formed by $OH^{\cdot}$ mediated hydroxylation of salicylate on spectrophotometer at 510 nm [8]. In another set of experiment, $OH^{\cdot}$ was generated non-enzymically by $FeSO_4$, sodium ascorbate, H_2O_2 and deoxyribose. After reaction in the absence or presence of root extract (50–500 µg), incubation mixture was assayed for malondialdehyde formed [9].

Statistical Analysis

The statistical analysis was done as before [5]. Similarly, the generations of oxygen free radicals with different concentrations of *A. indicus* root extract were compared with that of their formation without extract. The values were tested for significance at $P < 0.05$.

Results

Effect of *H. rosa sinensis* in Alloxan Induced Hyperglycemia

The acute administration of alloxan caused marked increase in their plasma levels of blood glucose 260 %, TC 57 %, TG 84 %, PL 45 %, FFA 49 % and lipid peroxide 229% following decrease in PHLA by 31 %. However, treatment with *H. rosa sinensis* root extract caused reversal in these levels of blood glucose by 30 %, TC by 24 %, PL

Table 1 Effect of *H. rosa sinensis* roots extract on blood glucose and serum lipid levels in alloxan induced diabetic rats

Groups	Blood glucose (mg/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipids (mg/dl)	FFA ($\mu\text{mol/l}$)	PHLA (nmol released/h/l)	Lipid peroxide (nmol MDA/dl)
Control	83.22 $\pm$ 9.67	83.98 $\pm$ 6.31	81.00 $\pm$ 7.77	69.19 $\pm$ 8.23	1.58 $\pm$ 0.17	16.00 $\pm$ 1.58	2.73 $\pm$ 0.49
Alloxan treated	300.23*** $\pm$ 32.20 (+260)	132.83*** $\pm$ 11.90 (+57)	149.29*** $\pm$ 9.27 (+84)	100.00*** $\pm$ 8.20 (+45)	2.36*** $\pm$ 0.30 (+49)	11.12*** $\pm$ 1.13 (–31)	8.98*** $\pm$ 1.40 (+229)
Alloxan + <i>Hibiscus rosa sinensis</i> extract (500 mg/kg bw)	220.41*** $\pm$ 20.40 (–30)	100.83*** $\pm$ 11.84 (–24)	24.43* $\pm$ 7.93 (–17)	76.29*** $\pm$ 7.74 (–23)	2.12 NS $\pm$ 0.25 (–10)	13.00* $\pm$ 0.83 (+17)	5.97*** $\pm$ 0.49 (–36)
Alloxan + glibenclamide (600 $\mu\text{g/kg}$ bw)	175.38*** $\pm$ 11.67 (–45)	98.36*** $\pm$ 17.05 (–26)	121.51* $\pm$ 12.35 (–19)	90.33 NS $\pm$ 7.86 (–8)	1.98** $\pm$ 0.29 (–20)	14.09*** $\pm$ 1.27 (+27)	5.55*** $\pm$ 0.99 (–39)

Alloxan treated diabetic group is compared with normal rats and alloxan + drug treated groups with diabetic group. Values in the parenthesis are % change
Values are expressed as mean $\pm$ SD of six rats * $P < 0.05$, ** $P < 0.001$, rest NS not significant

by 17 %, TG by 23 %, FFA by 10 %, lipid peroxide by 36 % and reactivation of PHLA by 17 %. The anti-diabetic and lipid lowering activities of *H. rosa sinensis* root extract was comparatively less to that of glibenclamide (Table 1).

Effect of *H. rosa sinensis* on Generation of Super Oxide Anions

The data in Table 2 showed that enzymic oxidation of xanthine to uric acid (A) as well as the generation of $\text{O}_2^{\cdot-}$ anions in xanthine–xanthine oxidase system, as measured by reduction of NBT to formazone (B) were inhibited to varying extents by root extract in a concentration dependent manner and this effect was maximum by 55 and 51 % respectively at 500 $\mu\text{g/ml}$ of test sample. The root extract also trapped the $\text{O}_2^{\cdot-}$ anions generated by non enzymic system of NADH–phenazine–methosulphate and were responsible for reduction of NBT in the reaction mixture. The effect was dose dependent and was highest by 58 % at 500 $\mu\text{g/ml}$ of test substance.

Effect of *H. rosa sinensis* on Generation of Hydroxyl Radicals

The data in Table 2 also showed that *H. rosa sinensis* root extract inhibited the formation of $\text{OH}^{\cdot}$ by enzymic system of hypoxanthine–xanthine oxidase and Fe^{2+} . Addition of extract (50–500 μg) inhibited the $\text{OH}^{\cdot}$ mediated formation of 2,3 dihydroxybenzoate in concentration dependant manner which was 50 % at 500 $\mu\text{g/ml}$ of test extract. Furthermore, this preparation, when added with reaction mixture containing Fe^{2+} –sodium ascorbate– H_2O_2 employed for nonenzymic generation of $\text{OH}^{\cdot}$ inhibited fragmentation of deoxyribose into MDA and this effect was maximum by 48 % at peak concentration (500 $\mu\text{g/ml}$) of root extract.

Discussion

In the present study, root of *H. rosa sinensis* was tested for its anti-diabetic, anti-dyslipidemic and anti-oxidant activities in alloxan induced diabetic rats. Alloxan causes reversible damage to insulin-producing β -cells found in the pancreas, and that is why this animal model have been used for primary screening of test drugs for antidiabetic activity [10]. We found that intoxication with alloxan caused increased levels of plasma glucose in rats and their reversal by the treatment with *H. rosa sinensis* root. Furthermore root extract also reduced lipid peroxide levels in above diabetic rats following inhibition of ROS generation in vitro.

Table 2 Effect of *H. rosa sinensis* roots extract on generation of oxygen free radicals in vitro

Concentration of <i>H. rosa sinensis</i> roots extract ($\mu\text{g/ml}$)	Generation of O_2^- anions			Generation of OH^- radicals		
	Enzymic system		Non enzymic system (NADH-PMS-NBT-system) ^b	Enzymic system (sodium salicylate-FeSO ₄ hypoXn-XnOD-system) ^c	Non enzymic system (FeSO ₄ -EDTA-H ₂ O ₂ -sodium ascorbate-deoxy ribose-system) ^d	
	(Xn-XnOD-system) ^a	(Xn-XnOD-NBT-system) ^b				
None	46.44 $\pm$ 1.3 8	118.05 $\pm$ 21.3 0	333.00 $\pm$ 16.8 2	540.09 $\pm$ 43.8 6	26.31 $\pm$ 2.22	
50	42.95 NS $\pm$ 0.95 (-8)	104.12 NS $\pm$ 11.82 (-12)	286.23 $\pm$ 21.43* (-14)	531.98 NS $\pm$ 10.28 (-1)	25.68 NS $\pm$ 1.26 (-2)	
100	40.11 * $\pm$ 1.22 (-14)	100.59 * $\pm$ 8.41 (-15)	239.99 $\pm$ 7.44** (-28)	500.69 $\pm$ 14.93* (-7)	21.00 $\pm$ 1.97** (-20)	
200	36.44 ** $\pm$ 0.98 (-22)	87.99 ** $\pm$ 3.89 (-25)	164.88*** $\pm$ 12.86 (-50)	419.33** $\pm$ 24.67 (-22)	17.44*** $\pm$ 0.78 (-34)	
250	32.18*** $\pm$ 0.56 (-31)	75.85*** $\pm$ 4.76 (-36)	160.99*** $\pm$ 4.98 (-52)	340.99*** $\pm$ 18.32 (-37)	15.98*** $\pm$ 1.34 (-39)	
300	28.34*** $\pm$ 0.46 (-39)	70.44*** $\pm$ 2.64 (-40)	151.78*** $\pm$ 11.87 (-54)	323.69*** $\pm$ 18.53 (-40)	15.11*** $\pm$ 2.44 (-42)	
400	23.88*** $\pm$ 2.66 (-49)	66.66*** $\pm$ 3.44 (-44)	148.88*** $\pm$ 12.69 (-55)	300.87*** $\pm$ 12.77 (-44)	14.96*** $\pm$ 0.59 (-43)	
500	20.98*** $\pm$ 1.22 (-55)	57.68*** $\pm$ 5.22 (-51)	139.48*** $\pm$ 12.13 (-58)	270.65*** $\pm$ 13.97 (-50)	13.65*** $\pm$ 1.33 (-48)	

Values are mean $\pm$ SD of four separate observations. The systems added with *H. rosa sinensis* root extract were compared with those without adding *Hibiscus rosa sinensis* root. Values in the parenthesis are % change * $P < 0.05$, *** $P < 0.01$; **** $P < 0.001$

^a nmol uric acid formed/min

^b nmol formazon formed/min

^c nmol 2,3 dihydroxy benzoate formed/h

^d nmol malondialdehyde formed/h

The phytochemical studies showed that *H. rosa sinensis* contains a variety of sterols, carbohydrates, glycosides, tannins, and flavonoids [11]. In addition to above mentioned constituents, the root bark of *H. rosa sinensis* also contains aliphatic enone ethers : oclade(-1)-yn-1-oic acid methyl ester 10-oxa, dec-9-ynoic acid methyl ester, non-8-ynoic acid methyl ester, nonadec-trans-10-enoic acid, 11-methoxy-9-oxo-methyl ester, octadec-11-ynoic acid, 10-oxo methyl ester, octadec-9-ynoic acid, 8-oxo methyl ester octadecanoic acid, 10-methylene-9-oxo methyl ester and cyclopropenoids [12]. It is suggested that all or some of these bioactive compounds may be responsible for hypoglycemic, antidyslipidemic and antioxidant effects of this herb. Recently, Mandade and Sreenivas [13] have reported hypoglycemic activity of aqueous ethanolic extract of aerial parts *H. rosa sinensis* in streptozotocin induced diabetic rats. Also Bhuvana et al. [14] showed that treatment with *H. rosa sinensis* flower petals (1,000 mg/kg of body weight) mixed with normal chow diet and administrated for 4 weeks, exhibited protective effects by reactivation of the antioxidant enzymes following a decrease in lipid peroxidation in 10 % D-glucose-induced oxidative stress in rat heart tissues. However, there are hardly any pharmacological investigations on root of this herb done so far. The present work is another report on new properties of *H. rosa sinensis* root to exert hypoglycemic, antidyslipidemic as well as antioxidant activities in in vivo and in vitro models. Further work on drug metabolism and to assess the biological activity of *H. rosa sinensis* root and its fraction is under progress to substantiate the present findings.

References

- Paranjpe P. Indian medicinal plants, forgotten healers: a guide to Ayurvedic herbal medicine. Delhi, India: Chaukhamba Sanskrit Pratishthan; 2001. p. 89–90, 102–3.
- Jadhav VM, Thorat RM, Kadam VJ, Sathe NS. *Hibiscus rosa sinensis* Linn “Rudrapusa”: a review. J Pharm Res. 2009;2(7): 1168–73.
- Stymvoli M, Goldstechn B, Haefn TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet. 2005;365:1333–45.
- Chattopadhyaya R, Pathak D, Jindal DP. Antihyperlipidemic agents. A review. Indian Drugs. 1996;33:85–97.
- Kumar V, Singh P, Chander R, Mahdi F, Singh S, Singh R, Khanna AK, Saxena JK, Mahdi AA, Singh VK. Hypolipidemic activity of *Hibiscus rosa sinensis* root in rats. Indian J Biochem Biophys. 2009;46(6):507–10.
- Bindoli A, Valante M, Cavallin L. Inhibition of xanthine oxidase and xanthine dehydrogenase activity. Pharmacol Res Commun. 1985;17:831–9.
- McCord JM, Fridovich IJ. Superoxide dismutase; an enzymic function for erythrocyte (hemocuprein). J Biol Chem. 1969;244: 6049–55.
- Richmond R, Halliwell R, Chauhan J, Darbre A. Superoxide dependent formation of hydroxyl radical by hydroxylation of aromatic compounds. Anal Biochem. 1981;118:320–35.

9. Halliwell B, Gutteridge JMC, Arora OI. The deoxyribose method: a simple test tube assay for determination of rate constants for reaction OH^- radicals. *Anal Biochem.* 1987;165:215–9.
10. Trivedi NA, Mazumdar B, Bhatt JD, Hemavathi KG. Effect of shilajit on blood glucose and lipid profile in alloxan induced diabetic rats. *Indian J Pharmacol.* 2004;36(6):373–6.
11. Gupta V, Bansal P, Garg A, Meena AK. Pharmacopoeial standardization of *Hibiscus rosa sinensis* Linn. *Int J Pharmaceut Clin Res.* 2009;1:124–6.
12. Nakatani M, Matsouka K, Uchio Y, Hase T. Two aliphatic enone ethers from *Hibiscus rosa sinensis*. *Phytochemistry.* 1994;35(5):1245–7.
13. Mandade R, Sreenivas SA. Anti-diabetic effects of aqueous ethanolic extract of *Hibiscus rosa sinensis* L. on streptozotocin-induced diabetic rats and the possible morphologic changes in the liver and kidney. *Int J Pharmacol.* 2011;3:363–9.
14. Bhuvana S, Mahesh R, Hazing-begum VM. Antioxidant activities of *Hibiscus rosa-sinensis* linn. During 10 % D-glucose feeding in rat heart tissues. *J Food Biochem.* 2011;35:792–802.