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Research Article

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Effect of 2-hydroxy 4- methoxy benzoic acid isolated from the roots of *Hemidesmus indicus* on blood glucose and tissue lipid peroxidation in streptozotocin-induced diabetic rats

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ABSTRACT

The aim of the present study was to investigate the effect of 2- Hydroxy 4- methoxy benzoic acid (HMBA) isolated from the roots of *Hemidemus indicus* on plasma glucose, serum α-amylase and α-glucosidase activity and on the increased levels of malondialdehyde (MDA), a marker of lipid peroxidation and on depleted levels of glutathione (GSH) in the liver, kidney and in the pancreas of streptozotocin-induced oxidative stress in diabetic rats. Treatment with HMBA (500 ìg/kg bw) for 7 weeks reduced the blood glucose level and the formation of MDA in the liver, kidney and pancreas. The activities of α-amylase and α-glucosidase were significantly (F>0.05; P<0.001) decreased on HMBA treatment. The GSH content was restored to the normal level of control group on HMBA treatment. Our results support that the antioxidant activity of HMBA occurs by quenching the lipid oxidation and restoring the GSH level. Based on the results HMBA have protective effect on liver, kidney and pancreas apart from hypoglycemic effect in STZ-induced oxidative stress.

Key words: 2- Hydroxy 4- methoxy benzoic acid, *Hemidemus indicus*, malondialdihyde, glutathione

INTRODUCTION

Diabetes is a progressive disease of carbohydrate metabolism and is one of the major killers in recent past. World Health Organization (WHO) suggests that worldwide the global population is in the midst of a diabetes epidemic with people in Southeast Asia and Western Pacific being mostly at risk. The number of cases of diabetes is currently 171 million is predicted to reach 366 million by the year 2030[1]. In diabetes the state of homeostasis of carbohydrate and lipid metabolism is improperly regulated by the pancreatic hormone, insulin; resulting in an increased blood glucose level. There are convincing experimental and clinical evidences that the generation of reactive oxygen species is increased in both type of diabetes and that the onset of diabetes is closely associated with oxidative stress[2] Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non ezymatic glycation of proteins[3] Abnormally high levels of free radicals and simultaneous decline of antioxidant defense systems can lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of complication of diabetes mellitus[4]

Plant extracts have long been used for the ethnomedical treatment of diabetes in various systems of medicine and are currently accepted as an alternative for diabetic therapy[5] The phytochemicals such as alkaloids, glycosides, galactomannan gun, polysaccharides, peptidoglycons, hypoglycans, guanidine, steroids, carbohydrates glycopeptides, terpenoids, amino acids and inorganic ions have been used to control diabetes[6] *Hemidesmus indicus* (Asclepiadaceae) is one of the indigenous Ayurvedic medicinal plants commonly available and widely distributed throughout India. The root bark of this plant has been used as a traditional medicine in the treatment of biliousness, blood diseases, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism[7]. *H. indicus* is also employed in traditional medicine for gastric ailments[8]. It mainly consists of essential oils and phytosterols such as hemidesmol, hemidesterol and saponins. It was already been reported that 2-hydroxy-4-methoxy benzoic acid (HMBA) exists in *H. indicus* having the molecular formula of $C_8H_8O_4$ [9].

HMBA is a white needle-shaped crystal which is soluble in water, methanol and chloroform and has a melting point of 155–158°C and lambda max 260 nm[9]. The presence of a benzene ring, methoxy group and hydroxyl group in the structure of HMBA was reported with the molecular weight of 168 [9]. The concentration of HMBA is in the range of 0.03–0.54% in the root bark of *H. indicus*[10]. HMBA is known to possess potent anti-inflammatory, antipyretic and antioxidant properties[11]. The compound effectively neutralizes viper-venom-induced changes in serum phosphatase and transaminase activity in male albino rats and is also known to reduce free radical formation[12]. The compound also has an adjuvant effect and antiserum potentiating activity against viper venom[13]. ACGIH, IARC, NIOSH, NTP and OSHA do not list it for carcinogenicity. The protective effect of *H.indicus* against rifampicin-and isoniazid induced hepatotoxicity in rats[14], as well as CCl_4 and paracetamol-induced hepatic damage[15], is known. Recently we have reported the antidiabetic activity of the aqueous extract of *H.indicus* in streptozotocin-induced diabetic rats [16]. In the present investigation HMBA was isolated from the roots of *H.indicus* and tested for their hypoglycemic and antiperoxidative activity in STZ-induced diabetic rats.

MATERIALS AND METHODS

Collection of Plants

The root of *H. indicus*, was collected from the Morappur

forest area, Dharmapuri District, Tamil Nadu, and authenticated by the forest department, where a voucher specimen was also submitted. Roots of *H. indicus* was washed with distilled water, shade dried, powdered and stored in an air- tight container until for further use.

Extraction of the pure compound

The root powder of *H. indicus* (100 g) was extracted with methanol using soxhlet apparatus and concentrated in rotary evaporator and then purified by silica gel (Merck,100 to 200 mesh) column chromatography using benzene-chloroform as eleuant[9] and the purity was checked by thin layer chromatography. The purity of the isolated compound was confirmed spectroscopically with the standard HMBA purchased from Sigma, USA.

Animals

Male Wistar rats weighing 150-200 g were used for this study. They were housed in standard environmental conditions (as per Institutional Animal Ethical Committee norms) and fed with standard pellet diet and water *ad libitum*.

Induction of diabetes

Diabetes was induced experimentally in rats by a single intraperitoneal injection of freshly prepared solution of streptozotocin (STZ) (Sigma, USA) at a dose of 35 mg/kg body weight in 0.1M citrate buffer, pH 4.5. The STZ treated animals were considered to be diabetic, if the blood glucose values were above 250 mg/dl and stabilized a period of 7 days and those animals alone were selected for this study.

Experimental protocol

Animals were divided in to four groups of six animals each. Group I served as a control; group II had STZ- treated surviving diabetic rats; group III served as a positive control and received a standard hypoglycemic agent, (100 mg/kg bw); group IV diabetic rats treated with the HMBA (500 ìg/kg bw) for 7 weeks by oral intubation method. Blood samples were collected at the end of the treatment period in heparinised vials for estimation of blood glucose[17] . The tissues, liver, kidney and pancreas were quickly removed from the sacrificed rat, placed in ice cold saline solution and trimmed of adipose tissue. Each tissue was finely minced and homogenized in 50 mM phosphate buffer, pH 7.4 and filtered using muslin cloth. Te supernatant was used for all the assays.

The activity of α-amylase was estimated by the method of Apostolides [18] and α-glucosidase was estimated by the method of Kim et al[19]. Malondialdehyde was measured by the spectroscopic method [20].Reduced glutathione (GSH) was determined by the method of Ellman [21].

Statistical analysis

Statistical analysis was performed using SPSS software package, version 9.05. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). All the results were expressed as mean ±SD for six rats in each group *P* values <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

The effect of HMBA on plasma glucose, α-amylase and α-glucosidase activity in STA-induced diabetic rats is given in Table 1. Oral administration of aqueous solution of HMBA significantly ($F>0.05$; $P<0.001$) reduced the blood glucose level when compared to untreated control rats. The activities of serum α-amylase and α-glucosidase were reduced significantly ($F>0.05$; $P<0.001$) to near-normal levels upon treatment with HMBA. In our study, diabetic rats had elevated level of blood glucose and treatment with HMBA reduced the blood glucose, which could not only be associated with increased insulin secretion but also delays the carbohydrate absorption as evidenced by the reduction in activities of α-amylase and α-glucosidase. These enzymes are responsible for absorption of glucose in the intestine.

The effect of HMBA on MDA levels of the liver, kidney and pancreatic tissue is given in Table 2. The elevated levels of MDA was decreased significantly ($F>0.05$; $P<0.001$) on HMBA treatment. Diabetes mellitus has been reported to generate reactive oxygen species (ROS). ROS can cause lipid peroxidation and there by elevation of lipid peroxidation markers (MDA). In the present study, the elevated levels of MDA in tissues of diabetic rats was in agreement with earlier reports[22, 23]. Treatment with HMBA reduced the levels of MDA to near normalcy, which could be associated with antioxidant potential of HMBA. Our report is consistent with the earlier reports that HMBA possess antioxidant activity [11].

The effect of HMBA on GSH content of the liver, kidney and pancreatic tissue is given in Table 3. The depleted levels of GSH was increased significantly ($F>0.05$; $P<0.001$) on HMBA treatment. Hyperglycemia can cause glycosylation of proteins and cellular lipid peroxidation, which, in turn, can cause depletion of reducing equivalents in the cell (GSH) [24]. The results of this study demonstrate that HMBA exhibits promising hypoglycemic activity and also helps to maintain GSH level by curbing ROS. Thus the plant-basedα-amylase and α-glucosidase inhibitor offers a prospective therapeutic approach for the management of diabetes mellitus.

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Table 1. Effect of HMBA on plasma glucose, serum a-amylase and a-glucosidase activity in STZ-induced diabetic rats

Groups	Dose (mg/kg/day)	Glucose (mg/dl)	α Amylase (U/L)	α -glucosidase (U/gm protein)
Normal	-	69.0±3.2 1	50±3.0	0.5±1.0
Diabetic control	-	268.0±3.1#	300±5.3#	2.00±2.0#
Glibenclamide	100	189.0±2.9 *	185±1.4*	1.0±1.2*
H.indicus	500	185.0±2.1 *	180±1.2*	0.9±1.4*
HMBA	0.5	153.0±2.5 *	160±1.3*	0.7±1.2*

Each value is mean ± SD for six rats in each group (n=6). Different from diabetic control, F > 0.05(ANOVA) and P< 0.05 (DMRT). # Different from normal control, F > 0.05(ANOVA) and P< 0.05 (DMRT).*

Table 2. Effect of HMBA on the liver, kidney and pancreatic malondialdehyde (MDA) content in STZ-induced diabetic rats

Groups	Dose (mg/kg/day)	Liver MDA (nmol/g wet weight)	Kidney MDA (µmol/L) (nmol/g wet weight)	Pancreas MDA (µmol/L) (nmol/g wet weight)
Normal	-	295.4±1.69	291.23±1.02	191.23±1.02
Diabetic control	-	375.32±2.20 #	365.23±3.65 #	255.19±2.65 #
Glibenclamide	100	245.65±1.02 *	300.95±1.24*	200.95±2.24*
H.indicus	500	200.21±1.54 *	280.56±1.58*	198.56±1.36*
HMBA	0.5	290.34±1.51*	292.02±1.7*	191.02±1.5*

Each value is mean ± SD for six rats in each group (n=6), Different from diabetic control, F > 0.05 (ANOVA) and P< 0.05 (DMRT). # Different from untreated control, F > 0.05 (ANOVA) and P< 0.05 (DMRT*

Table 3. Effect of HMBA on liver, kidney and pancreatic tissue GSH content in STZ-induced rats

Groups	Dose (mg/kg/day)	Liver GSH (µmol/L)	Kidney GSH (µmol/L)	Pancreas GSH (µmol/L)
Normal	-	45.50±1.02	56.3±1.01	46.3±1.01
Diabetic control	-	40.56±1.06 #	52.4±1.23 #	42.4±1.23 #
Glibenclamide	100	34.7±1.05 *	46.2±1.24*	36.2±1.14*
H.indicus	500	39.24±1.21	41.6±1.35*	39.6±1.15*
HMBA	0.5	42.5±1.6*	49.0±1.5*	40.0±1.4*

Each value is mean ± SD for six rats in each group (n=6), Different from diabetic control, F > 0.05 (ANOVA) and P< 0.05 (DMRT). # Different from untreated control, F > 0.05 (ANOVA) and P< 0.05 (DMRT).*

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