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Influence of Tetrahydrocurcumin on Hepatic and Renal Functional Markers and Protein Levels in Experimental Type 2 Diabetic Rats

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Abstract: Curcumin is the most active component of turmeric. It is believed that curcumin is a potent antioxidant and anti-inflammatory agent. Tetrahydrocurcumin is one of the major metabolites of curcumin that exhibits many of the same physiologic and pharmacological activities as curcumin and in some systems may exert greater antioxidant activity than curcumin. Oral administration of tetrahydrocurcumin at 80 mg/kg body weight to diabetic rats for 45 days resulted in a significant reduction in blood glucose and significant increase in plasma insulin levels. In addition, the diabetic rats had decreased levels of plasma total protein, albumin, globulin and albumin/globulin ratio as compared to control rats. After treatment with tetrahydrocurcumin and curcumin total protein, albumin, globulin and albumin/globulin ratio were brought back to near normal. The activities of hepatic and renal markers were significantly elevated in diabetic rats as compared to control rats, and treatment with tetrahydrocurcumin and curcumin has reversed these parameters to near normal levels. In diabetic rats, the decreased levels of urea, uric acid and creatinine with increased levels of albumin and urine volume was observed, and treatment with tetrahydrocurcumin and curcumin reversed these parameters to near normal. Tetrahydrocurcumin appeared to have a better protective effect when compared to curcumin.

Diabetes mellitus is by far the most common of endocrine disorders and a major threat to health care worldwide. The increase of free radical–mediated toxicity is well documented in streptozotocin-diabetic rats. The liver is the main effector organ for maintaining plasma glucose levels within narrow limits. Hyperglycaemia can generate a redox imbalance inside the cells, especially in the liver [1]. A model antidiabetic drug should possess both hypoglycaemic and antioxidant properties, without any adverse effects. Plant drugs are frequently considered to be less toxic than synthetic ones [2].

The liver and kidney play a major role in the pathogenesis of type 2 diabetes. The liver enzymes aspartate transaminase, alanine transaminase, alkaline phosphatase and γ-glutamyl transpeptidase are used routinely for assessing liver function. Although they are present in tissues throughout the body, they are most often elevated in patients with liver diseases or high alcohol consumption. Nephrotoxicity is one of the major side effects of drug therapy in clinical practice, frequently leading to acute renal failure. Many physiological mechanisms have been implicated in streptozotocin-induced renal injury in diabetes [3].

Curcumin (diferuloylmethane) is the substance that gives the spice turmeric, which is extensively used in Indian cuisine as a component of curry powder, providing its yellow colour. It is believed that curcumin is a potent antioxidant and anti-inflammatory agent. Curcumin has been shown to reduce the hyperlipidaemia [4], delay the development of cataract [5], ameliorate renal lesions [3] and reduce cross-linking of collagen [6] in a streptozotocin-treated diabetic animal model. Curcumin has also been shown to lower blood glucose levels in type 2 diabetic KK-Ay mice [7] and streptozotocin-treated rats [8].

Tetrahydrocurcumin is one of the major metabolites of curcumin, with potential bioactivity. This metabolite was identified in intestinal and hepatic cytosol from human beings and rats [9]. Recently, attention has been focused on tetrahydrocurcumin, as one of the major metabolites of curcumin, because this compound appears to exert greater antioxidant activity in both *in vitro* and *in vivo* systems [10]. Structurally, tetrahydrocurcumin and curcumin have identical β-diketone structures and phenolic groups, but differ in that tetrahydrocurcumin lacks the double bonds [10]. Sugiyama et al. [11] demonstrated that tetrahydrocurcumin exhibited similar physiological and pharmacological properties as the active form of curcumin *in vivo*. Naito et al. [9] showed clear involvement of tetrahydrocurcumin in biochemical and molecular actions at the cellular level in ameliorating oxidative stress in cholesterol-fed rabbits. In our previous study, we have demonstrated the antidiabetic effect of tetrahydrocurcumin in streptozotocin-induced diabetic rats [12].

To our knowledge, no other biochemical investigations have so far been carried out on the effect of tetrahydrocurcumin in hepatic and renal functional markers and protein level status of experimental diabetic rats. The present investigation was carried out to study the effect of tetrahydrocurcumin on hepatic and renal functional markers and protein levels in rats with streptozotocin- and nicotinamide-induced diabetes.

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Table 1.

Effect of tetrahydrocurcumin (THC) on blood glucose, insulin, total protein, albumin, globulin and A/G ratio in plasma of control and experimental animals.

Groups	Fasting blood glucose (mg/dl)	Plasma insulin (μU/ml)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control rats	94.28 ± 6.45*	12.05 ± 0.74*	6.99 ± 0.41*	3.71 ± 0.22*	3.28 ± 0.19*	1.13 ± 0.01*
Diabetic control	283.25 ± 8.75†	3.98 ± 0.21†	4.04 ± 0.27†	1.88 ± 0.12†	2.16 ± 0.15†	0.87 ± 0.04†
Diabetic + THC (80 mg/kg)	114.45 ± 7.35‡	9.21 ± 0.58‡	6.50 ± 0.34‡	3.43 ± 0.15‡	3.07 ± 0.19‡	1.17 ± 0.01‡
Diabetic + Curcumin (80 mg/kg)	129.15 ± 8.21§	8.17 ± 0.38§	5.93 ± 0.33§	3.10 ± 0.17§	2.83 ± 0.16§	1.09 ± 0.01§

Values are given as mean ± S.D. for six rats in each group. A/G ratio, albumin/globulin ratio.
Values not sharing a common superscript symbol differ significantly at $P < 0.05$ (Duncan's multiple range test).

Materials and Methods

Animals. The study was performed on adult male albino rats of the Wistar strain weighing 180–220 g. According to the experimental protocol approved by the Committee for Research and Animal Ethics of Annamalai University, animals were housed in cages and maintained in 24 ± 2°C normal temperature and a 12-hr light:dark cycle. The animals were fed on pellet diet (Lipton India Ltd., Mumbai, India) and water *ad libitum*.

Drugs and chemicals. Tetrahydrocurcumin was a gift provided by Sabinsa Corporation (Piscataway, NJ, USA). Curcumin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and biochemicals were of analytical grade.

Induction of diabetes. Type 2 diabetes mellitus was induced [13] in overnight fasted rats by a single intraperitoneal injection of 65 mg/kg body weight of streptozotocin, 15 min. after the intraperitoneal injection administration of 110 mg/kg body weight of nicotinamide. Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in saline. Hyperglycaemia was confirmed by elevated glucose levels in plasma, determined at 72 hr and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl will be used for the study.

Experimental design. For the experiment, 24 rats were divided into four groups of six rats each, after the induction of streptozotocin diabetes. The experimental period was 45 days. Group 1: normal untreated rats. Group 2: diabetic control rats. Group 3: diabetic rats given tetrahydrocurcumin (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days. Group 4: diabetic rats given curcumin (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days.

At the end of 45 days, the animals were deprived of food overnight and killed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose. Plasma was separated for the estimation of insulin. Collection of urine by using metabolic cages animals kept in overnight.

Analytical methods. Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd., Baroda, India) [14]. Plasma insulin was assayed by ELISA using a Boehringer-Mannheim kit with an ES300 Boehringer analyser (Mannheim, Germany).

Total protein and albumin was estimated by using a reagents kit (Qualigens diagnostics, Baroda, India) according to biuret and bromocresol green dye-binding methods. Serum enzymatic activities of aspartate transaminase, alanine transaminase, alkaline phosphatase and γ-glutamyl transpeptidase were determined on fully automated chemistry analyser Roche/Hitachi-912 (Roche Diagnostics, Mannheim, Germany) using Roche Diagnostics GmbH kits. The values were expressed as IU/l serum.

The levels of urea, ureic acid, creatinine, albumin and total protein were estimated spectophotometrically according to the standard procedures using commercially available diagnostic kits (Sigma Diagnostics (I) Pvt Ltd.).

Statistical analysis. The data for various biochemical parameters were analysed using ANOVA, and the group means were compared by Duncan's multiple range test. Values were considered statistically significant if $P < 0.05$ [15].

Results

Table 1 shows the level of blood glucose and insulin, total protein, albumin, globulin and albumin/globulin ratio in the plasma of different experimental groups. The diabetic control rats showed a significant increase in the level of blood glucose with significant decrease in the level of plasma insulin. Oral administration of tetrahydrocurcumin to diabetic rats significantly reversed the above biochemical changes. The diabetic rats had decreased levels of plasma total protein, albumin, globulin and albumin/globulin ratio when compared to control rats. After treatment with tetrahydrocurcumin and curcumin total protein, albumin, globulin and albumin/globulin ratio were brought back to near normal levels. The protective effect of tetrahydrocurcumin was more prominent compared with curcumin.

Table 2 represents the effect of tetrahydrocurcumin and curcumin on changes in the activities of serum aspartate transaminase, alanine transaminase, alkaline phosphatase, γ-glutamyl transpeptidase, urea, uric acid and creatinine of control and experimental rats. The activities of hepatic markers were significantly elevated in diabetic rats when compared to control rats. Tetrahydrocurcumin and curcumin treatment to diabetic rats reversed the above changes in a significant manner when compared to diabetic control rats. In our study, the levels of urea, uric acid and creatinine are elevated remarkably in the serum of diabetic rats as compared to control rats. Diabetic rats treated with tetrahydrocurcumin and curcumin showed the reversed of these parameters near normal. The effect of tetrahydrocurcumin was more potent than that of curcumin.

Table 3 shows urine output and the levels of urea, uric acid, creatinine and albumin in the urine of diabetic rats. Diabetic rats have decreased levels of urea, uric acid, creatinine and increased levels of albumin was observed, and treatment

Table 2.

Effect of tetrahydrocurcumin (THC) on serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), urea, ureic acid and creatinine in control and experimental diabetes.

Groups	Control rats	Diabetic control	Diabetic + THC (80 mg/kg)	Diabetic + Curcumin (80 mg/kg)
Hepatic functional markers				
AST (IU/l)	74.61 ± 5.08*	121.02 ± 7.14†	83.53 ± 6.10‡	94.18 ± 5.36§
ALT (IU/l)	27.73 ± 1.89*	64.54 ± 3.81†	34.22 ± 2.50‡	40.08 ± 2.28§
ALP (IU/l)	77.63 ± 5.28*	146.24 ± 8.63†	86.55 ± 6.32‡	93.19 ± 5.30§
GGT (IU/l)	11.80 ± 0.81*	27.84 ± 1.65†	14.69 ± 1.07‡	18.94 ± 1.08§
Renal functional markers				
Urea (mg/dl)	23.20 ± 1.27*	39.52 ± 2.71†	25.96 ± 1.15‡	29.11 ± 1.68§
Ureic acid (mg/dl)	1.18 ± 0.07*	2.32 ± 0.16†	1.28 ± 0.08‡	1.40 ± 0.07§
Creatinine (mg/dl)	0.95 ± 0.05*	2.32 ± 0.16†	1.08 ± 0.06‡	1.25 ± 0.05§

Values are given as mean ± S.D. for six rats in each group.
Values not sharing a common superscript symbol differ significantly at $P < 0.05$ (Duncan's multiple range test).

with tetrahydrocurcumin and curcumin has reversed these parameters to near normal levels.

Discussion

New drugs are investigated in animals both for desired effects and for the undesired (toxic) effects. This is important because any hepatic and renal damage will alter structure and function of these vital organs and have serious effects on overall metabolism. The liver is the most important organ in the metabolism of drugs and other substances. Liver cell destruction shows its effects mostly as important in the liver cell membrane permeability, which results in the leaking out of tissue content into the blood stream [16]. In several organs, cell membrane damage is followed by release of a number of cytoplasmic enzymes to the blood, a phenomenon that provides the basis for clinical diagnosis. Abnormal levels in serum of aspartate transaminase, alanine transaminase and alkaline phosphatase are of clinical and toxicological importance, being indicative of tissue damage by toxicants or disease condition [17].

Insulin generally has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation [18]. A previous report has shown that protein synthesis is decreased in all tissues due to decreased production of alkaline phosphatase in absolute or relative deficiency of insulin [19] that may be responsible for decreased level of plasma protein; albumin and globulin may be related with increased level of plasma insulin in diabetic treated with tetrahydrocurcumin and curcumin. Rasch and Mogensen [20] have reported that the plasma albumin/globulin ratio was lower in diabetic animals. Increased protein catabolism in diabetic might have induced a direct adverse effect on the synthesis and secretion of albumin. Diabetic rats treated with tetrahydrocurcumin and curcumin, brought back albumin/globulin ratio also to near normal results.

The changes in serum enzyme activities are normal in uncomplicated diabetes. However, when tissue damage caused by metabolic and circulatory alterations occur, their activities are increased. This suggests that liver and kidney damage, primarily caused by congestion and metabolic disorders might be the cause of these enzymatic changes [21]. Elevated activities of serum transaminases are a common sign of hepatic dysfunction, and are more frequently observed among people with diabetes, than in the general population. Furthermore, diabetic complications such as limited joint mobility, retinopathy and neuropathy are associated with liver enzyme activities, independent of alcohol consumption, body mass index and metabolic control of diabetes [17].

In our study, the activities of serum transaminases were found to be elevated in diabetic rats. In this context, several investigators reported increases in aspartate transaminase and alanine transaminase in the liver and serum of

Table 3.

Effect of tetrahydrocurcumin (THC) on urea in urine, ureic acid, creatinine and albumin, urine volume in control and experimental diabetes.

Groups	Urea in urine (mg/dl)	Ureic acid (mg/dl)	Creatinine (mg/dl)	Albumin (μg/dl)	Urine volume (ml/day)
Control rats	146.78 ± 8.72*	7.97 ± 4.74*	2.85 ± 0.17*	149.31 ± 7.26*	9.38 ± 0.56*
Diabetic control	108.65 ± 7.46†	5.75 ± 0.39†	1.73 ± 0.12†	314.88 ± 15.62†	21.69 ± 1.49†
Diabetic + THC (80 mg/kg)	136.15 ± 8.65‡	7.53 ± 0.39‡	2.60 ± 0.16‡	164.38 ± 9.35*‡	11.19 ± 0.71‡
Diabetic + curcumin (80 mg/kg)	112.43 ± 7.05§	7.03 ± 0.12§	2.38 ± 0.13§	180.03 ± 9.81‡	12.51 ± 0.68§

Values are mean ± S.D. for six rats in each group.
Values not sharing a common superscript symbol differ significantly at $P < 0.05$ (Duncan's multiple range test).

streptozotocin-diabetic rats [17]. The changes in levels of serum enzymes are directly related to changes in the metabolism in which the enzymes are involved. The increased protein catabolism accompanying gluconeogenesis in the diabetic state might be the reason for the elevated activities of these enzymes, which were brought back to near normal by tetrahydrocurcumin treatment. This result shows the normalizing effects of tetrahydrocurcumin on hepatocellular damage and suppression of gluconeogenesis.

The observation of an increased serum activity of alkaline phosphatase in diabetes has been interpreted as a manifestation in serum of the increased phoshatase activity that may occur in tissues in the diabetic state [22]. In this condition, increased activity has been reported for glucose-6-phosphatase and fructose-1,6-bisphosphatase in the liver. These phosphatases are enzymes distinct from alkaline phosphatase. Belfiore et al. [23] also reported that, owing to some overlapping substrate specificity shown by the phosphatases, and the possibility that an enhanced alkaline phosphatase might be present in tissues of diabetic patients, it can also be ruled out that phosphatases released from tissues, mainly liver, might contribute to the elevated serum alkaline phosphatase activity [21].

The activity of serum alkaline phosphatase was observed to increase in streptozotocin-diabetic rats. In support of our finding, it has been found that the liver was necrotized in streptozotocin-diabetic rats [24]. Therefore, the increase of the activity of alkaline phosphatase in serum is mainly due to the leakage of the enzyme from the liver into circulation [24]. On the other hand, the administration of tetrahydrocurcumin to diabetic rats reduced alkaline phosphatase activity towards normal.

γ-Glutamyl transpeptidase has a key role in amino acid transport across the membrane and catalyses the initial step in the breakdown of glutathione (i.e. transfer of γ-glutamyl moiety of glutathione to a variety of amino acids and peptides [25]). γ-Glutamyl transpeptidase is a cytoplasmic enzyme found in very high concentrations in the liver. Elevation of serum γ-glutamyl transpeptidase activity is generally regarded as one of the most sensitive indices of hepatic damage [17]. A highly significant elevation in the activity of γ-glutamyl transpeptidase was observed in serum of diabetic rats. This is in accordance with earlier investigations, which have demonstrated a dramatic increase in γ-glutamyl transpeptidase expression in the liver of diabetic rats. Elevated activity of γ-glutamyl transpeptidase in serum takes place as a result of oxidative insult in the liver [26]. In addition, hepatocellular damage or cholestasis may also contribute to the elevation in the activity. Increased activity of γ-glutamyl transpeptidase in streptozotocin-induced diabetic rats was lowered to near normal by tetrahydrocurcumin treatment that indicates possible prevention of necrosis by tetrahydrocurcumin treatment.

In accordance with the above findings, streptozotocin-induced diabetes plays a significant role in the alteration of liver functions because the activities of aspartate transaminase, alanine transaminase, alkaline phosphatase and γ-glutamyl transpeptidase were significantly increased. However, tetrahydrocurcumin treatment of diabetic rats significantly reduced the activities of serum enzymes. We have previously reported that tetrahydrocurcumin treatment, as there was no significant change in the activities of serum enzymes in normal rats [27], can be stated that the drug is non-toxic to the mammalian system.

Urea is the major nitrogen-containing metabolic product of protein metabolism; uric acid is the major product of purine nucleotides, adenosine and guanosine. The diabetic hyperglycaemia and creatinine are considered as significant markers of renal function [28]. The increased urea, uric acid and creatinine and increased urine output indicate kidney dysfunction in diabetic rats. Treatment with tetrahydrocurcumin resembles the present result. Treatment with tetrahydrocurcumin reversed these parameters to near normal which could be due to decreased metabolic disturbances of other pathway such as protein and nucleic acid metabolism as evidenced by improved glycaemic control. The clinical manifestation of diabetic nephropathy is the development of microalbuminuria. Gomes et al. [29] observed that developed albuminuria may be due to impaired tubular reabsorption or leakage of albumin due to damaged glomerular membrane, which leads to alterations in size or changes in selective barriers of the glomeruli.

In conclusion, our study suggests that liver and kidney functions are highly altered in diabetic state. Treatment with tetrahydrocurcumin and curcumin reversed these changes in diabetic rats, which indicates that tetrahydrocurcumin and curcumin protect the hepatic and renal function in the diabetic condition.

References

1 Gallou G, Ruelland A, Legras B, Maugendre D, Allanic H, Cloarec L. Plasma MDA in type 1 and 2 diabetes. Clin Chim Acta 1993;**214**:227–34.

2 Ramesh B, Pugalendi KV. Impact of umbelliferone (7-hydroxycoumarin) on hepatic marker enzymes in streptozotocin diabetic rats. Indian J Pharmacol 2006;**38**:209–10.

3 Babu P, Srinivasan K. Amelioration of renal lesions associated with diabetes by dietery curcumin in streptozotocin induced diabetic rats. Mol Cell Biochem 1998;**181**:87–96.

4 Babu P, Srinivasan K. Hypolipidemic action of curcumin, the active principle of turmeric (*Curcuma longa*) in streptozotocin induced diabetic rats. Mol Cell Biochem 1997;**166**:169–75.

5 Suryanarayana P, Saraswat M, Mrudula T, Krishna P, Krishnaswamy K, Reddy G. Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. Invest Ophthalmol Vis Sci 2005;**46**:2092–9.

6 Sajithlal GB, Chithra P, Gowri C. Effect of curcumin on the advanced glycation and cross-linking of collagen in diabetic rats. Biochem Pharmacol 1998;**56**:1607–14.

7 Nishiyama T, Mae T, Kishida H, Tsukagawa M, Mimaki Y, Kuroda M. Curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa* L) suppress and increase in blood glucose level in type 2 diabetic KK-Ay mice. J Agric Food Chem 2005;**53**:959–63.

8 Mahesh T, Balasubashini M, Menon V. Effect of photo-irradiated curcumin treatment against oxidative stress in streptozotocin-induced diabetic rats. J Med Food 2005;**8**:251–5.

9 Naito M, Wu X, Normura H, Kodama M, Kato M, Osaswa T. The protective effect of tetrahydrocurcumin on oxidative stress in cholesterol-fed rabbits. J Atheroscler Thromb 2002;**9**:243–50.
10 Okada K, Wangpoengtrakul C, Tanaka T, Toyokuni S, Uchida K, Osawa T. Curcumin and especially tetrahydrocurcumin ameliorate oxidative stress-induced renal injury in mice. J Nutr 2001;**131**:2090–5.
11 Sugiyama Y, Kawakishi S, Osawa T. Involvement of the β-diketone moiety in the antioxidant mechanism of tetrahydrocurcuminoids. Biochem Pharmacol 1996;**52**:519–25.
12 Pari L, Murugan P. Effect of tetrahydrocurcumin on blood glucose, plasma insulin and hepatic key enzymes in streptozotocin induced diabetic rats. J Basic Clin Physiol Pharmacol 2005;**16**:257–74.
13 Masiello P, Broca C, Gross R et al. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 1998;**47**:224–9.
14 Lott JA, Turner K. Evaluation of trinder's glucose oxidase method for measuring glucose in serum and urine. Clin Chem 1975;**21**:1754–60.
15 Duncan BD. Multiple range tests for correlated and heteroscedastic means. Biometrics 1957;**13**:359–64.
16 Ozsoy-Sacan O, Yanardag R, Orak H, Ozgey Y, Yarat A, Tunali T. Effects of parsley (*Patroselinum crispum*) extract versus glibornuride on the liver of streptozotocin-induced diabetic rats. J Ethanopharmacol 2006;**104**:175–81.
17 Singh SN, Vats P, Suri S et al. Effects of an antidibetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats. J Ethnopharmacol 2001;**76**:269–77.
18 Murray RK, Granner DK, Mayesa PA. Harper's Biochemistry, 25th edn. Appleton and Lange, Stamford, CT, 2000.
19 Chatterjee MN, Shinde K. Text Book of Medical Biochemistry. Metabolism of Carbohydrate. Jaypee Brothers Medical Publishers Private Ltd., New Delhi, India, 1994.
20 Rasch R, Mogensen CE. Urinary excretion of albumin and total protein in normal and streptozoyocin-diabetic rats. Acta Endocrinol 1980;**95**:376–81.
21 Geidam MA, Adoga GI, Sanda FA. Effects of aqueous stem bark extract of *Cissus populnea* on some serum enzymes in normal and alloxan induced diabetic rats. Pak J Biol Sci 2004;**7**:1427–9.
22 Belfiore F, Napoli E, Vecchio L. Increased activity of some enzymes in serum in cases of severely decompensated diabetes with and without ketoacidosis. Clin Chem 1972;**18**:1403–6.
23 Belfiore F, Vecchio LL, Napoli E. Serum enzymes in diabetes mellitus. Clin Chem 1973;**19**:447–52.
24 Mansour HA, Newairy A, Yousef SA, Sheweita MI. Biochemical study on the effects of some Egyptain herbs in alloxan-induced diabetic rats. Toxicology 2002;**170**:221–8.
25 Meister A, Anderson ME. Glutathione. Annu Rev Biochem 1983;**52**:711–60.
26 Whitfield JB. Gamma glutanyl transferase. Crit Rev Clin Lab Sci 2001;**38**:263–355.
27 Pari L, Murugan P. Tetrahydrocurcumin: effect on chloroquine-mediated oxidative damage in rat kidney. Basic Clin Pharmacol Toxicol 2006;**99**:329–34.
28 Perone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function. New insights into old concepts. Clin Chem 1992;**38**:1933–53.
29 Gomes MB, Lucctetti MR, Gonacalvez MFR. Influence of first morning urine volume, fasting blood and glycosylated Hb on first morning urine albumin excretion. Braz J Med Biol Res 1997;**30**:191–6.

