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EFFECT *CASSIA AURICULATA* ON LIPID PEROXIDATION AND LIPIDS IN STREPTOZOTOCIN - NICOTINAMIDE INDUCED DIABETIC RATS

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Abstract

Introduction: The present study investigated the beneficial effect of Tanner's cassia *Cassia auriculta* flower extract (CFEt) and leaf extract (CLEt) on lipid peroxidation and lipids properties in streptozotocin (STZ) –induced diabetic rats.

Methods: The effects of an aqueous extract of CFEt (0.45 g/kg), CLEt (0.45 g/kg) and glybenclamide on plasma glucose, insulin, thiobarbituric acid reactive substances (TBARS) and hydroperoxides formation, cholesterol, triglycerides, free fatty acids and phospholipids, histopathological examination of liver and kidney section of normal and experimental groups.

Results: Oral administration of CFEt and CLEt (0.45 g/kg) aqueous extract and glibenglamide to diabetic rats for 45 days significantly resulted in significant reduction in blood glucose and significant increase in plasma insulin levels. In addition, CFEt at (0.45 g/kg) and CLEt (0.45 g/kg) significantly decreased the levels of serum and tissue lipids as compared to untreated diabetic rats, with significant decrease in thiobarbituric acid reactive substances (TBARS) and hydroperoxides formation in serum, liver and kidney, suggesting its role in protection against lipid peroxidation induced membrane damage.

Conclusion: CFEt and CLEt has beneficial effect on plasma insulin and blood glucose level. Moreover it was a prevention of lipid metabolism defects could represent a protective mechanism against the development of atherosclerosis. The CFEt administration showed more effective than CLEt and glibenclamide.

Key words: lipids, lipid peroxidation, *Cassia auriculta* flower extract, *Cassia auriculta* leaf extract

Introduction

Diabetes is a metabolic disease and its incidence is considered to be high all over the world (Devendra and Eisenbarth, 2003). Epidemiological studies and clinical studies strongly support the notion that hyperglycemia is the principal cause of complications. Effective blood glucose control is the key for preventing or reversing diabetic complications and improving quality of life in patients with diabetes (Zimmet et al. 2001). Thus, sustained reduction in hyperglycemia will decrease the risk of developing microvascular and macrovascular complications (Brownlee, 2001). Throughout the world many traditional plant treatments for diabetes exist. However, few have received scientific or medical scrutiny and the WHO has recommended accordingly that traditional

plant treatment for diabetes warrant further evaluation (WHO, 1980). Preliminary report indicates blood glucose lowering effect of *cassia auriculata* (Pari and Murugan, 2007). This section focuses on antihyperglycemic effect of aqueous, ethanolic and chloroform extracts of CFEt and CLEt in STZ diabetic rats.

Management of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use the natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs are having undesirable side effects (Kameswara Rao and Appa Rao 2001). The plants with antidiabetic activities provide useful sources for the development of drugs in the treatment of diabetes mellitus. Medicinal plants with hypoglycemic activity were used for many centuries and some times as regular constituents of the diet, it is assumed that they do not have many side effects (Halim Eshart 2002). Phytochemicals isolated from plant source are used for the prevention and treatment of cancer, heart disease, diabetes, high blood pressure etc. (Mary et al. 2002).

Cassia auriculata L. (Ceasalpiniaceae) is a shrub that has attractive yellow flowers, commonly used for the treatment of skin disorders and body odour. It is a native plant present in different parts of India. Indigenous people use various parts of the plant for diabetes mellitus (Murugan, 2010). It is widely used in Ayurvedic medicine as a "Kalpa drug" which contains five parts of the shrub (roots, leaves, flowers, bark and unripe fruits) which are taken in equal quantity, dried and then powdered to give "Avarai Panchaga Choornam", for the control of sugar levels and reduction of symptoms such as polyuria and thirst in diabetes (Brahmachari and Augusti, 1961; Shrotri and Aiman, 1963). A literature survey showed that a decoction of leaves, flowers, and seeds of the *Cassia auriculata* mediate an antidiabetic effect (Shrotri and Aiman, 1963; Murugan,2015a). Thus, the available reports show that very little work has been done with respect to *Cassia auriculata* flowers, other than its hypoglycemic effects. In our previous study, we have demonstrated the antidiabetic effect of *Cassia auriculata* flower extract (CFEt) in STZ induced diabetic rats [Murugan,2015b]

Materials and methods

Chemicals

Stereptozotocin was obtained from Himedia Laboratory Limited, Mumbai, India. All other reagents used were of analytical grade.

Plant Material

Cassia auriculata flowers were collected freshly from Neyveli, Cuddalore District, Tamil Nadu, India. The plant was identified and authenticated at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (No.231) was deposited in the Botany Department of Annamalai University.

Preparation of plant extract

Five hundred g of *Cassia auriculata* flowers were extracted with 1,500 ml of water by the method of continuous hot extraction at 60ºC for six hours and evaporated. The residual extract was dissolved in water and used in the study (Jain, 1968).

Induction of diabetes

Non-Insulin dependent diabetes mellitus was induced (Masiello et al. 1998) in overnight fasted rats by a single intraperitonial injection of 65 mg/kg streptozotocin, 15 min after the i.p administration of 110 mg/kg of nicotinamide. Streptozototocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl were used for the study.

Experimental procedure

In the experiment, a total of 30 rats (24 diabetic surviving rats, six normal rats) were used. The rats were divided in to six groups of six rats each.

Group 1: Normal untreated rats.

Group 2: Diabetic control rats given 1 ml of aqueous solution daily using an intragastric tube for 45 days.

Group 3: Diabetic rats given CFEt (0.45 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45 days.

Group 4: Diabetic rats given CLEt (0.45 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45days.

Group 5: Diabetic rats given glibenclamide (600 μg/ kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45days.

At the end of 45 days, the animals were deprived of food overnight and sacrificed 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 and other biochemical parameters. Liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood. The tissues were weighed and 10% tissue homogenate was prepared with 0.025 M Tris - HCl buffer, pH 7.5. After centrifugation at 200 rpm for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances and hydroperoxides. For the determinations of lipids the liver and kidney tissues were weighed and lipids were extracted from tissues by the method of Folch et al. (1957) using chloroform - methanol mixture ($CHCl_3$:MeOH)(2:1 v/v).

Analytical procedure

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) (Lott and Turner 1975). Plasma insulin was assayed by ELISA using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany). Haemoglobin was estimated using the cyanmethaemoglobin method described by Drabkin and Austin (1932). Glycosylated haemoglobin was estimated according to the method of Sudhakar Nayak and Pattabiraman (1981) with modifications according to Bannon (1982).

Thiobarbituric acid reactive substances were measured by the method of Fraga et al. (1988). Hydroperoxides were determined by the method of Jiang et al. (1992).

Extraction of lipids from serum and tissues was carried out according to the procedure of Folch *et al*. (1957) by using chloroform - methanol (2:1 v/v) mixture. From this, the total cholesterol, triglycerides, free fatty acids and phospholipids were estimated by the method of Zlatkis *et al*. (1953), Foster and Dunn (1973) Falholt *et al*. (1973) and Zilversmit and Davis (1950), respectively.

Histopathological study

The liver and kidney samples fixed for 48h in 10% formal-saline were dehydrated by passing successfully in different mixture of ethyl alcohol – water, cleaned in xylene and embedded in paraffin. Sections of liver and kidney (4-5 □m thick) were prepared and then stained with hematoxylin and eosin dye, which mounted in neutral deparaffinated xylene (DPX) medium for microscopic observations.

Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Duncan's multiple range test (DMRT). Values were considered statistically significant if $p < 0.05$ (Duncan 1957).

Results

Table 1 shows the level of blood glucose, total haemoglobin, glycosylated haemoglobin and plasma insulin of different experimental groups. There was a significant elevation in blood glucose level, whereas plasma insulin levels decreased significantly in streptozotocin diabetic rats, compared with

normal rats. The effect of CFEt was more prominent when compared with CLEt and glibenclamide. The diabetic control rats showed a significant decrease in the level of total haemoglobin and significant increase in the level of glycosylated haemoglobin. Oral administration of CFEt and CLEt to diabetic rats significantly restored total haemoglobin and glycosylated haemoglobin levels. In the case of normal rats, the level of haemoglobin and glycosylated haemoglobin remained unaltered.

Table 2 represents the concentration of TBARS and hydroperoxides in tissues of normal and experimental rats. There was a significant elevation in tissue TBARS and hydroperoxides during diabetes, when compared to the corresponding normal group. Administration of CFEt, CLEt and glibenclamide significantly decreased the lipid peroxidation in diabetic rats. The CLEt was more potent than CLEt and glibenclamide.

The levels of serum and tissues cholesterol, free fatty acids, triglycerides and phospholipids of normal and experimental rats are given in table 3. Cholesterol, free fatty acids, triglycerides and phospholipids were significantly decreased in CFEt and CLEt treated rats as compared to diabetic rats. Oral administration of CFEt at (0.45 g/kg) and CLEt (0.45 g/kg) significantly decreased the levels of serum and tissue lipids as compared to untreated diabetic rats. The CFEt administration showed more effective than CLEt and glibenclamide.

Histopathological observations in liver, kidney and pancreas of control and experimental rats

Liver

Pathological changes of liver include hepatic nuclear condensation portal triad with inflammation, and sinusoidal dilation in diabetic control rats. The above pathological changes were remarkably reduced in rats treated with CFEt, CLEt and glibenclamide, with mild inflammation and mild sinusoidal dilatation.

Kidney

Diabetic control rat kidney showed glomeruli mesangial capillary proliferation with tubular epithelial damage. These changes were reduced in CFEt, CLEt and glibenclamide treated diabetic rats. Tissues of normal rats treated with CFEt and CLEt revealed near normal appearance with no significant changes.

Pancreas

Histopathological studies (compared to normal) show the atrophic acini and damaged islet cells in diabetic rat pancreas and these changes were markedly reduced in diabetic rats treated with CFEt, CLEt and glibenclamide.

Discussion

Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes (Gandhi, 2001). In rats, administration of diabetogenic doses of STZ induces alterations in lipids. The results of our present study clearly show that *Scoparia dulcis* plant extract has a lowering action on serum TG, TC, VLDL-C, LDL-C, FFA and PL. There is substantial evidence that lowering the TC, particularly LDL-C level will lead to a reduction in the incidence of coronary heart disease (CHD), which is still a leading cause of death in diabetic patients. Reduction of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease (Bishayee and Chatterjee, 1994).

The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of FFA from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depot (Al-Shamaony et al. 1994).

A decrease in serum TC level with an increase in the HDL-C level in diabetic rats treated with CFEt and CLEt can be ascribed to the reduction of LDL-C and VLDL-C. VLDL-C is biosynthesized in liver and secreted into the blood and transformed into intermediate density lipoprotein (IDL) by

lipoprotein lipase (Shigematsu et al. 2001). Low VLDL-C levels in serum induced by CFEt and CLEt may be due to (a) repression of hepatic synthesis of VLDL-C (b) elevation of fatty acid oxidation (c) inhibition of VLDL-C secretion from the liver. Studies on IDDM and STZ-induced diabetes in experimental rats have suggested that an increase in circulatory VLDL-C and their associated TG are largely due to defective clearance of these particles from circulation (Suresh Babu and Srinivasan, 1997). As there is a close relationship between elevated serum TC level and the occurrence of atherosclerosis, the ability of the extract in selective reduction of TC through the reduction of VLDL-C and LDL-C components could be beneficial in preventing atherosclerotic conditions and thereby reduce the possibility of CHD.

As regards to the effect of the extract on serum HDL-C, our results clearly show that the level of this lipoprotein fraction increased with CFEt and CLEt treatment. Epidemiological studies have established the important role of HDL-C level as a protective factor against CHD (Gordon et al. 1977) and it is now considered as the main 'antiatherogenic' lipoprotein (Siddique et al. 1987). The exact role of HDL-C in lipid transport is not clear. It is considered that HDL-C is involved in the transport of cholesterol from peripheral tissues into the liver (Segal et al. 1984). However, in our observation an improvement of HDL-C level with a concomitant rise in the ratio of HDL-C to TC (expressed in terms of AAI) following the plant extract treatment was observed.

A marked increase in serum TG, FFA and fluctuations in serum TC was observed in uncontrolled diabetic rats. Excess fatty acids in serum produced by the STZ-induced diabetes promote the conversion of some liver fatty acids into PL and TC. These two substances along with excess TG formed at the same time in the liver may be discharged into the blood in the form of lipoproteins (Bopanna et al. 1997). It has been reported that the plasma lipoproteins increase as much as 3 fold in STZ-induced diabetes giving a total concentration of serum lipids of several percent rather than normal. This high lipid concentration may lead to the rapid development of atherosclerosis in diabetic patients (Pushparaj et al. 2000). Besides serum TC, the elevated levels of TG and PL were also significantly counteracted by CFEt and CLEt.

HMG-CoA reductase catalyzes the rate-limiting step in cholesterol biosynthesis and its activity correlates closely with the rate of tissue TC synthesis. Increased activity of HMG-CoA reductase activity was observed in our study. Decreased HMG-CoA/Mevalonate ratio indicates increased activity of the enzyme. Several reports have shown increased activity of HMG-CoA reductase in diabetic rats (Murugan, 2015c). The increase in the liver TC in diabetic control rats observed in our study could be due to increased cholesterogenesis. The significant increase in the level of extrahepatic TC could be due to decreased removal of TC from extrahepatic tissues by HDL-C.

The increased concentration of FFA in liver and kidney may be due to lipid breakdown and this may cause increased microsomal lipid peroxidation. Liver and kidney PL was increased in diabetic control rats. PL is present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core (Cohn and Roth, 1996). PL is vital part of biomembrane rich in PUFA, which are susceptible substrate for free radicals such as $O_2^{\bullet-}$ and $OH^{\bullet}$ radicals (Ahmed et al. 2001). Accumulation of TG is one of the risk factors in CHD. The significant increase in the level of TG in liver and kidney of diabetic control rats may be due to the lack of insulin. Since under normal condition, insulin activates the enzyme lipoprotein lipase and hydrolysis TG (Frayn, 1993). CFEt and CLEt reduces TG in plasma and tissues of STZ-induced diabetic rats and may prevent the progression of CHD.

Conclusion

It can be concluded from the data that CFEt and CLEt significantly reduces the level of serum and tissue lipids and lipid peroxidation marker, which are actively raised in streptozotocin diabetic rats. CFEt and CLEt has beneficial effect on plasma insulin and blood glucose level. Moreover it was a prevention of lipid metabolism defects could represent a protective mechanism against the development of atherosclerosis. The CFEt administration showed more effective than CLEt and glibenclamide.

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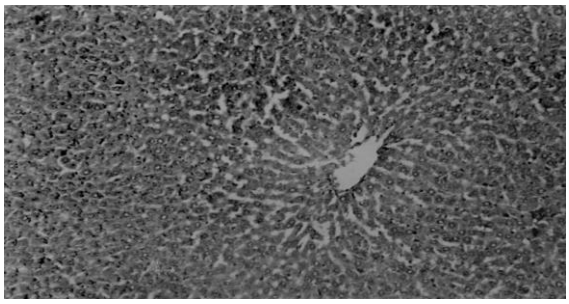

Figure1 A. Control rat liver.
Normal architecture of liver.

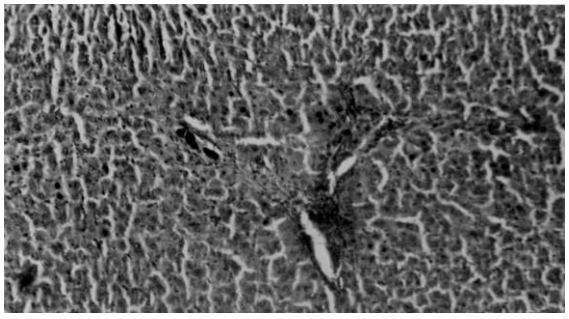

Figure 1B. Diabetic rat liver. Hepatocytic nuclear condensation portal triad with inflammation, sinusoidal dilation.

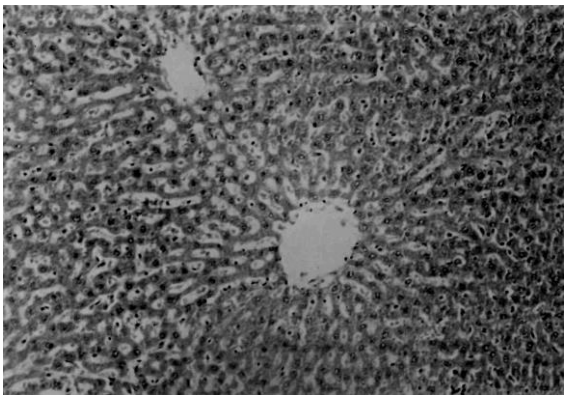

Figure 1C.Diabetic + CFEt (0.45 g/kg) treated rat liver. Normal appearance of liver cells.

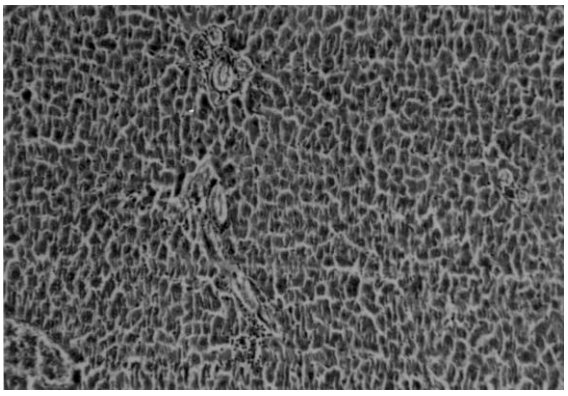

Figure 1D. Diabetic+ + CLEt (0.45 g/kg) treated rat liver. Normal hepatocytes with mild inflammation.

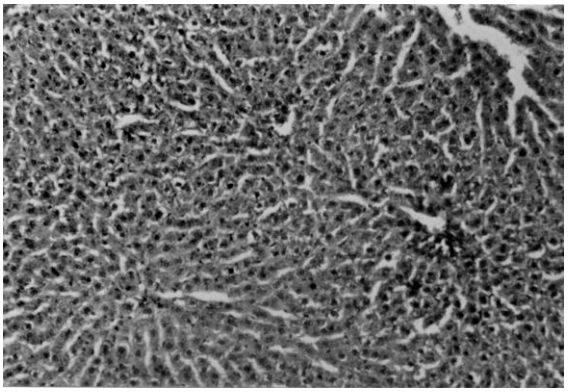

Figure 1E. Diabetic+ glibenclamide treated rat liver. Normal hepatocytes with mild inflammation and mild sinusoidal dilation.

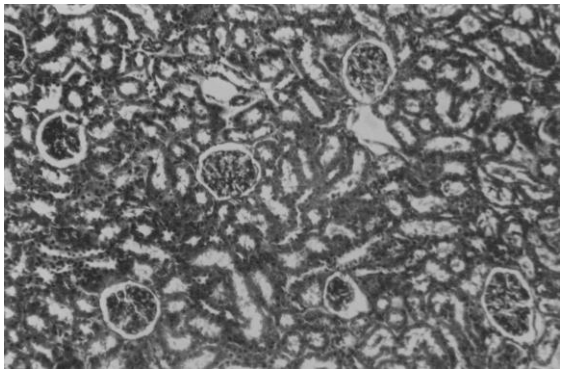

Figure 2A. Control rat kidney.
Normal architecture of kidney.

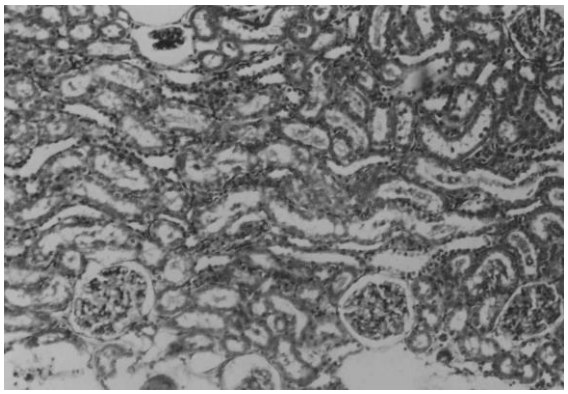

Figure 2B. Diabetic rat Kidney. Glomeruli mesangial capillary proliferation with tubular epithelial damage.

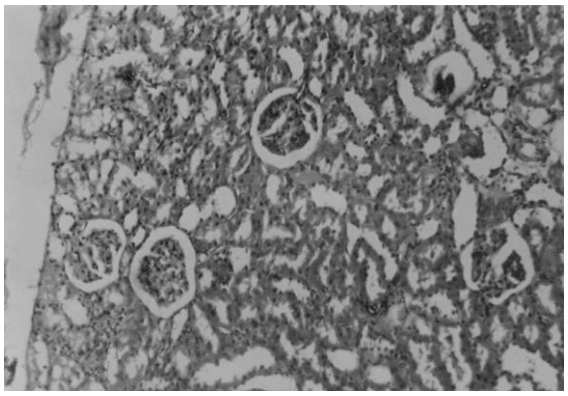

Figure 2C. Diabetic + CFEt (0.45 g/kg) treated rat kidney. Normal glomeruli with tubules.

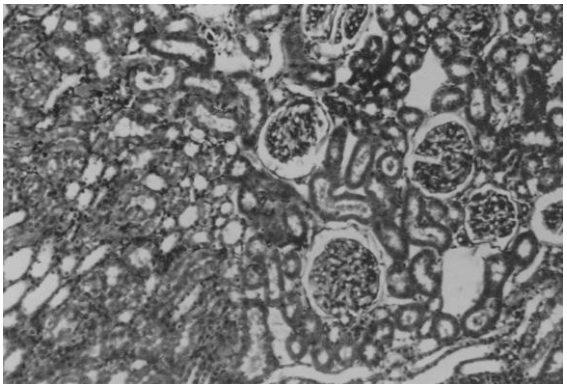

Figure 2D. Diabetic+ + CLEt (0.45 g/kg) treated rat kidney. Normal tubules with focal glomerular changes.

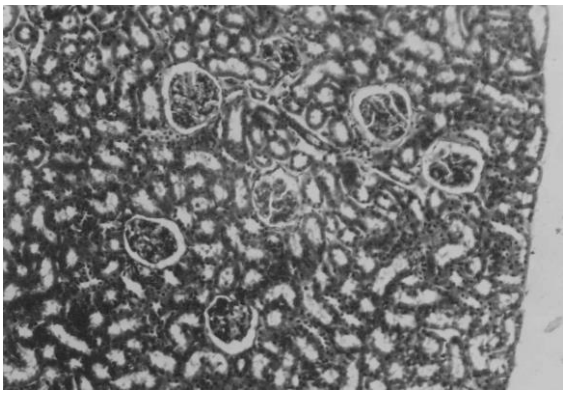

Figure 2E. Diabetic+ glibenclamide treated rat kidney. Normal tubules with focal glomerular changes.

Table 1. Effect of CFEt and CLEt on the levels of blood glucose, plasma insulin, haemoglobin and glycosylated haemoglobin in normal and experimental rats

Groups	Fasting blood glucose (mg/dl)	Plasma insulin (μU/ml)	Total haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g Hb)
Normal	99.53 ± 5.12^{a}	12.65 ± 0.53^{a}	12.21 ± 0.54^{a}	0.31 ± 0.03^{a}
Diabetic control	293.41 ± 7.52^{b}	3.92 ± 0.34^{b}	8.54 ± 0.35^{b}	0.79 ± 0.04^{b}
Diabetic+CFEt (0.45g/kg)	114.21 ± 6.25^{c}	9.92 ± 0.75^{c}	11.65 ± 0.66^{c}	0.42 ± 0.03^{c}
Diabetic+CLEt (0.45g/kg)	135.54 ± 6.63^{d}	8.52 ± 0.33^{d}	10.98 ± 0.58^{d}	0.47 ± 0.03^{d}
Diabetic+ Glibencalamide (600 μg/ mg)	142.87 ± 7.51^{d}	8.87 ± 0.35^{d}	10.87 ± 0.45^{d}	0.52 ± 0.04^{d}

Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Table 2. Influence of CFEt and CLEt on the content of TBARS and hydroperoxides in rats liver and kidney.

Groups	Normal	Diabetic control	Diabetic+CFEt (0.45g/kg)	Diabetic+CLEt (0.45g/kg)	Diabetic+ Glibencalamide (600 μg/ kg)
TBARS					
Liver (mM/100g tissue)	0.79 ± 0.05^{a}	1.88 ± 0.11^{b}	1.42 ± 0.04^{c}	1.17 ± 0.03^{d}	1.23 ± 0.03^{d}
Kidney (mM/100g tissue)	1.82 ± 0.13^{a}	3.94 ± 0.23^{b}	2.31 ± 0.12^{c}	1.99 ± 0.15^{d}	2.18 ± 0.03^{e}
Hydroperoxides					
Liver (mM/100g tissue)	82.15 ± 5.25^{a}	105.43 ± 5.73^{b}	94.84 ± 4.53^{c}	91.35 ± 4.54^{ac}	87.54 ± 0.05^{d}
Kidney (mM/100g tissue)	59.12 ± 3.15^{a}	81.53 ± 4.54^{b}	71.58 ± 4.47^{c}	63.45 ± 4.24^{c}	72.23 ± 4.25^{d}

Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (Duncan's Multiple Range Test).

Table 3. Effect of CFEt and CLEt **on the levels of cholesterol, free fatty acids, triglycerides and phospholipids in serum, liver and kidney of normal and experimental rats.**

Groups	Normal	Diabetic control	Diabetic+CFEt (0.45g/kg)	Diabetic+CLEt (0.45g/kg)	Diabetic+ Glibencalamide (600 μg/ kg)
Cholesterol					
Serum (mg/100ml)	87.51 ± 5.32^{a}	170.65 ± 12.02^{b}	109.54 ± 7.25^{c}	129.25 ± 6.25^{d}	139.54 ± 6.35^{d}
Liver (mg/100g wet tissue)	325.77 ± 14.25^{a}	515.35 ± 32.52^{b}	419.54 ± 23.25^{c}	449.25 ± 27.58^{c}	449.45 ± 27.55^{c}
Kidney(mg/100g wet tissue)	375.32 ± 22.67^{a}	535.12 ± 30.15^{b}	415.85 ± 20.34^{c}	445.24 ± 25.15^{c}	455.62 ± 26.13^{c}
Free fatty acids					
Serum (mg/100ml)	79.32 ± 4.45^{a}	142.77 ± 10.52^{b}	88.54 ± 5.15^{c}	106.21 ± 5.66^{d}	119.54 ± 6.47^{d}
Liver (mg/100g wet tissue)	575.35 ± 30.74^{a}	825.45 ± 45.15^{b}	710.65 ± 40.32^{c}	778.16 ± 35.26^{c}	775.11 ± 35.21^{c}

Kidney(mg/100gwet tissue)	410.52 ± 19.54^{a}	600.31 ± 39.65^{b}	535.61 ± 34.62^{c}	565.21 ± 25.25^{d}	515.13 ± 20.15^{d}
Triglycerides					
Serum (mg/100ml)	57.21 ± 3.35^{a}	92.32 ± 7.21^{b}	63.51 ± 4.21^{c}	77.25 ± 4.47^{d}	81.32 ± 4.25^{d}
Liver (mg/100g wet tissue)	335.58 ± 18.35^{a}	620.32 ± 35.45^{b}	418.32 ± 18.89^{c}	510.32 ± 20.77^{d}	500.11 ± 25.77^{d}
Kidney(mg/100gwet tissue)	265.54 ± 17.12^{a}	410.25 ± 21.12^{b}	365.32 ± 25.88^{c}	430.42 ± 17.17^{d}	435.42 ± 17.11^{d}
Phospholipids					
Serum (mg/100ml)	115.67 ± 6.56^{a}	175.12 ± 13.12^{b}	130.31 ± 8.34^{c}	108.32 ± 8.65^{d}	157.650 ± 8.54^{d}
Liver (g/100g wet tissue)	1.57 ± 0.10^{a}	3.12 ± 0.16^{b}	1.91 ± 0.15^{c}	2.19 ± 0.13^{d}	2.25 ± 0.12^{d}
Kidney (g/100g wet tissue)	1.59 ± 0.13^{a}	2.30 ± 0.13^{b}	1.75 ± 0.13^{c}	1.96 ± 0.12^{d}	1.99 ± 0.13^{d}

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).