CHAPTER III

RESULTS & DISCUSSIONS

1. The hypoglycemic effect of crude water extract from *C. fenestratum* (CE) in normal male Wistar rats.

1.1 A Single-oral dose of CE in the glucose tolerance test (OGTT)

The effect of a single-oral dose of CE on blood glucose concentration in normal male Wistar rats had been studied. Treatment group 1, 2, 3, 4 and 5 have been given CE orally at doses 0.10, 0.25, 0.50, 0.75 or 1 g/kg body weight, respectively; and control group fed distilled water, at 30 min before glucose feeding. CE-treated rats showed significant decrease ($p<0.05$) in blood glucose concentration when compared with control group at 30 min after glucose feeding (Figure 13). Blood glucose concentration of treatment group 1 to 5 were 162.20 ± 7.33, 161.18 ± 6.67, 161.47 ± 3.60, 160.07 ± 4.35 and 154.33 ± 5.14 mg/dl, respectively; and control group was 181.03 ± 3.85 mg/dl. Percentage decrease was 10.40, 10.97, 10.80, 11.57 and 14.75 %, respectively. The highest dose in this experiment, 1 g/kg body weight, could significantly reduce ($p<0.01$) blood glucose levels which remained reduced for a period of 180 min.

The crude water extract might contain compounds that could suppress glucose level. It is possible that the aqueous extract may act at a peripheral level. If so, there might be facilitating glucose metabolism via the increase of its cellular uptake by increase of the number of receptors and/or the glucose receptor affinity. Furthermore, it may also be possibly performed at the hepatic level that glycogenesis increase via the activation of glycogen synthetase (Naik *et al.*, 1991; Benwahhoud *et al.*, 2001).

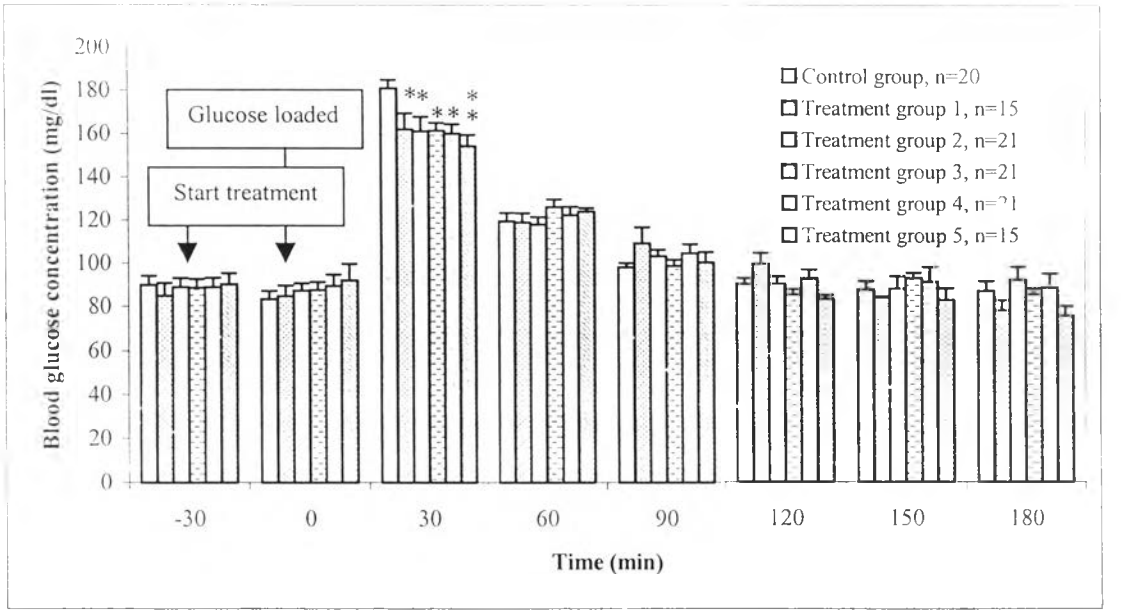

Figure 13. Effect of oral CE on blood glucose concentration in normal male Wistar rats; 3 hr-duration.

Each bar represents Mean ± S.E.M.

Treatment group 1: Normal rats fed CE at 0.10 g/kg body weight.

Treatment group 2: Normal rats fed CE at 0.25 g/kg body weight.

Treatment group 3: Normal rats fed CE at 0.50 g/kg body weight.

Treatment group 4: Normal rats fed CE at 0.75 g/kg body weight.

Treatment group 5: Normal rats fed CE at 1 g/kg body weight.

Control group: Normal rats fed distilled water at 1ml/kg body weight.

* Significant at $p < 0.05$, compared to control group.

** Significant at $p < 0.01$, compared to control group.

1.2 Repeated-oral doses of CE in normal rats.

The results of oral repeated-doses of CE in normal rats are illustrated in Figure 14-17. Rats group 1 received CE at dose 1 g/kg body weight /day and group 2 were fed distilled water. There was no significant difference ($p>0.05$) of body weight (Figure 14), relative body weight gained (Figure 15) and relative liver weight (Figure 16) between group 1 and group 2.

Rats were given CE or distilled water once daily for 14 days and the fasting blood glucose concentrations for at least 6 hr were determined on day 7 and 14. Blood glucose concentration of rats group 1 and group 2 on day 7 was 119.60, 115.33 mg/dl and on day 14 was 137.33, 141.40 mg/dl, respectively; the results showed no significant difference (Figure 17). This result suggests no hypoglycemic effect in normal rats. Therefore, oral repeated-doses of CE in normal rats which had normal β cell did not decrease blood glucose levels due to the fact that in normal rats carbohydrate metabolism may be stabilized by body mechanism (Murray *et al.*, 2000).

On day 14, rat blood samples were collected for determination of blood clinical biochemistry and hematologic analysis. Enzyme levels in serum were shown in Table 6. AST, ALT, ALP of CE-treated and control group, there were no significant difference in mean values of serum enzyme level between group 1 and group 2 of the three enzymes, AST, ALT and ALP. AST and ALT activities in serum of the normal animals remain unchanged in liver though AST and ALT activities were slightly less than that of control. However, AST values was slightly higher than the normal value of 99.47 ± 10.1 U/L (Appendix I) (Inala *et al.*, 2001a). ALP activities were higher than that of control. Daniels (2002) reported that mildly elevated levels are found with viral hepatitis, growing children, large doses of vitamin D and leukemia. Therefore, CE-treating in rats showed no adverse effect on liver function after feeding at a dose of 1 g/kg body weight/day for 14 days.

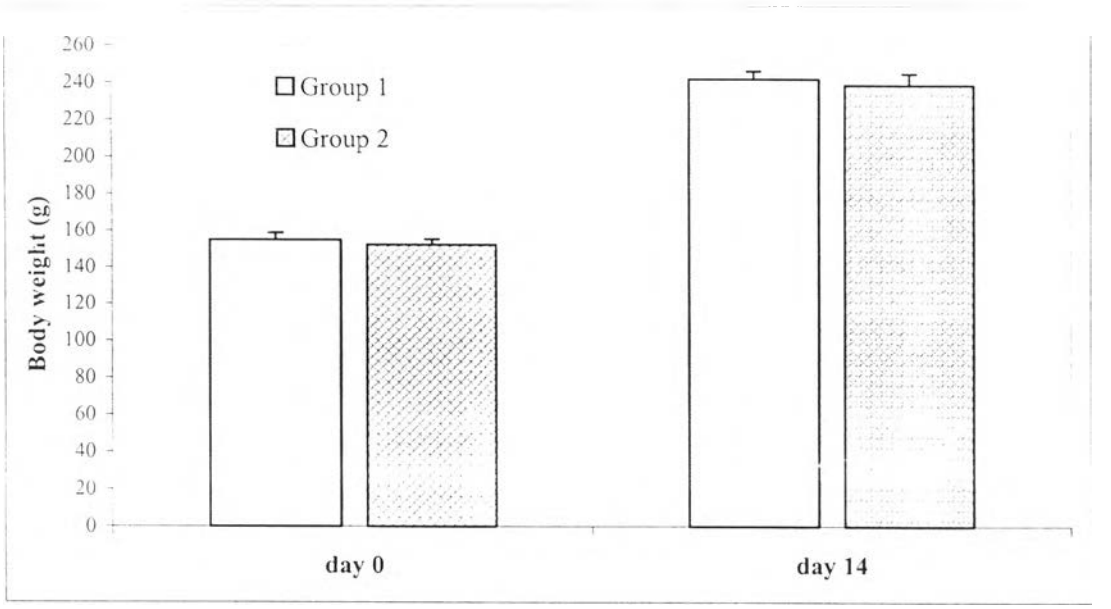

Figure 14. Body weight of rats on day 0 and day 14 after daily oral repeated-doses of CE in normal male Wistar rats.

Each bar represents Mean ± S.E.M. Each group of 10 rats.

Group 1: Normal rats fed CE at 1 g/kg body weight/day, for 14 days.

Group 2: Normal rats fed distilled water.

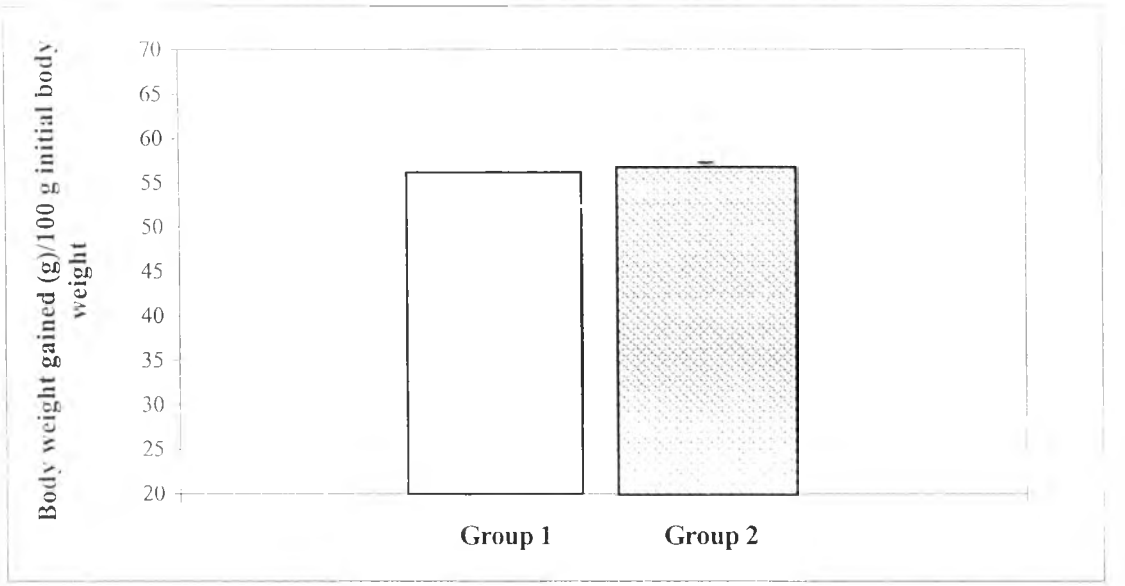

Figure 15. Percentage of relative body weight gained on day 14 after daily oral repeated-doses of CE in normal male Wistar rats.

Each bar represents Mean ± S.E.M. Each group of 10 rats.

Group 1: Normal rats fed CE at 1 g/kg body weight/day, for 14 days.

Group 2: Normal rats fed distilled water.

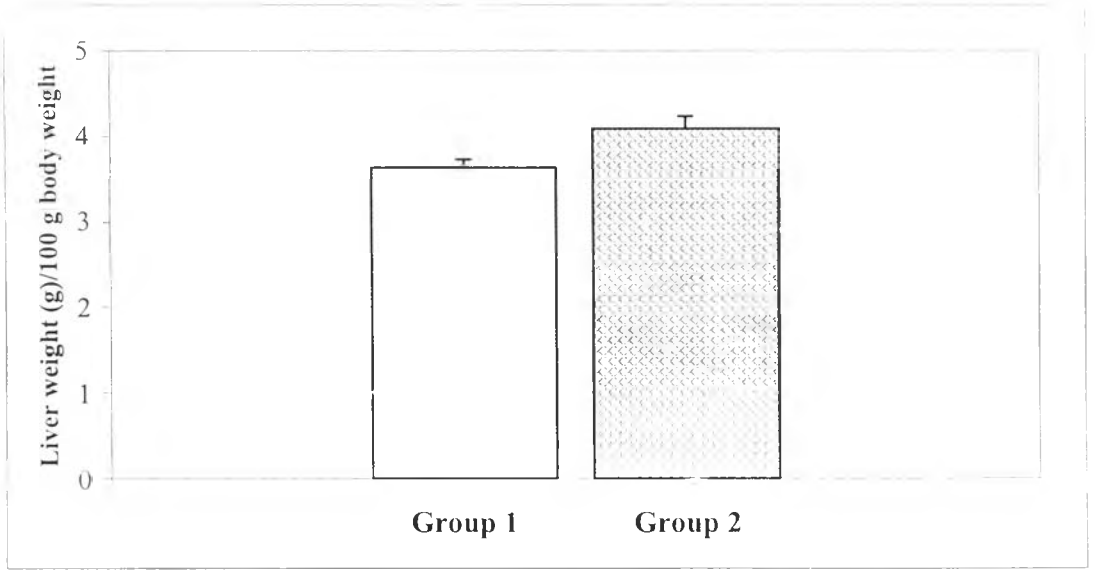

Figure 16. Relative liver weight on day 14 after daily oral repeated-doses of CE in normal male Wistar rats.

Each bar represents Mean ± S.E.M. Each group of rats.

Group 1: Normal rats fed CE at 1 g/kg body weight/day, for 14 days.

Group 2: Normal rats fed distilled water.

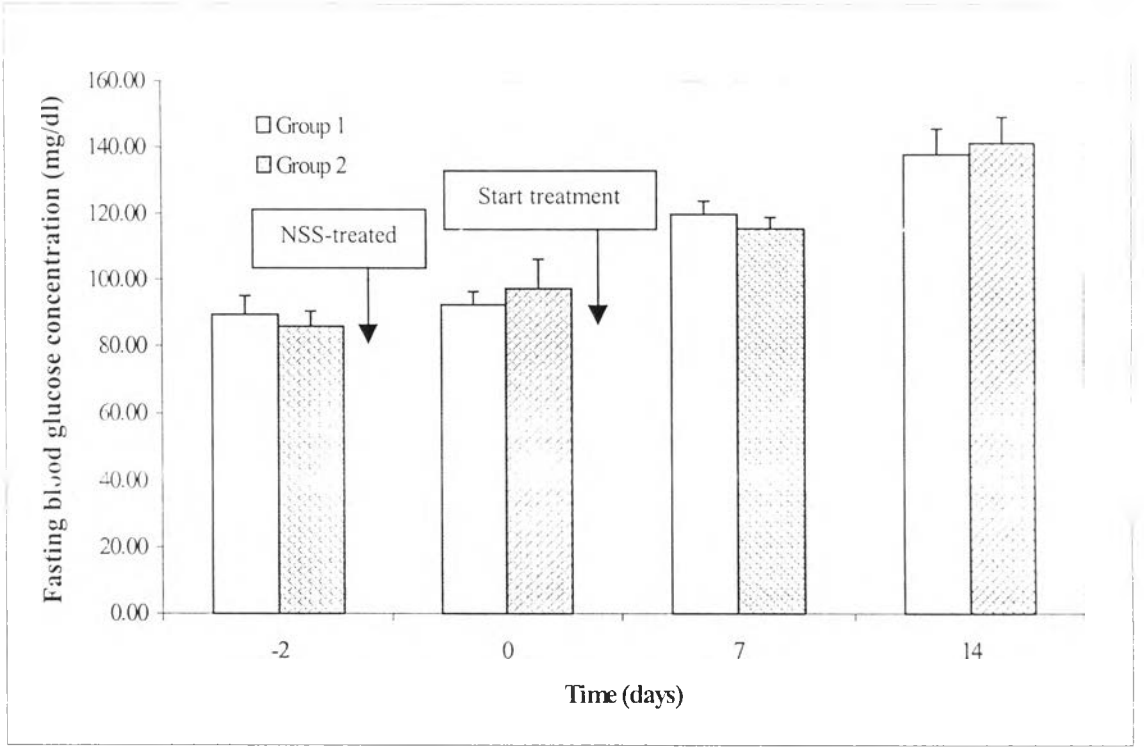

Figure 17. Effect of CE on fasting blood glucose concentration in normal male Wistar rats treated for 7 and 14 days.

Each bar represents Mean ± S.E.M., NSS = normal saline solution. Rats were fasted at least 6 hr before blood samples were collected. Each group of 10 rats.

Group 1: Normal rats fed CE at 1 g/kg body weight/day, for 14 days.

Group 2: Normal rats fed distilled water.

Table 6. Level of serum enzymes in normal male Wistar rats after daily feeding CE or distilled water for 14 days.

Group	N	Body weight (g)	AST (units)	ALT (units)	ALP (units)
1	10	241.94 ± 4.54	116.40 ± 11.40	41.50 ± 10.65	428.00 ± 14.81
2	10	238.52 ± 4.31	140.80 ± 30.29	48.58 ± 7.59	393.16 ± 33.07

Data are Mean ± S.E.M.

Group 1: Normal rats fed CE at 1 g/kg body weight/day, for 14 days.

Group 2: Normal rats fed distilled water.

Table 7. Biochemical analysis of serum in normal male Wistar rats after daily feeding CE or distilled water for 14 days.

Group	Glucose (mg%)	Cholesterol (mg%)	Triglyceride (mg%)	BUN (mg%)	Creatinine (mg%)
1	137.70 ± 7.77	35.90 ± 3.86	121.00 ± 9.75	23.03 ± 1.02	0.61 ± 0.13
2	141.14 ± 7.89	49.50 ± 6.00	110.75 ± 11.28	22.24 ± 0.81	0.55 ± 0.04

Data are Mean ± S.E.M. Each group of 10 rats.

Group 1: Normal rats fed CE at 1 g/kg body weight/day, for 14 days.

Group 2: Normal rats fed distilled water.

Table 8. Hematological parameters of normal male Wistar rats after daily feeding CE or distilled water for 14 days.

Group	RBC (Cell x 10^6 / mm^3)	Hb (%)	Hct (%)	WBC (Cell x 10^3 / mm^3)	Neu (%)	Band (%)	Eos (%)	Baso (%)	Lymp (%)	Mono (%)
1	5.35 ± 0.24	11.25 ± 0.48	34.78 ± 1.92	18.78 ± 0.57	5.89 ± 1.5	---	0-1	---	92.73 ± 1.25	---
2	5.87 ± 0.34	12.23 ± 0.85	42.53 ± 3.17	18.65 ± 1.25	6.20 ± 0.95	---	0-2	---	92.88 ± 0.23	---

Data are Mean ± S.E.M. Each group of 10 rats.

Group 1: Normal rats fed CE at 1 g/kg body weight/day, for 14 days.

Group 2: Normal rats fed distilled water.

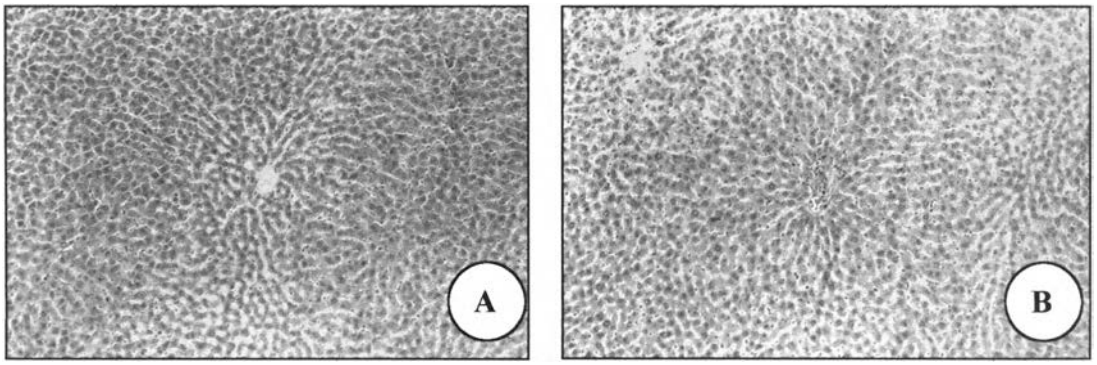

Figure 18. Liver, Hematoxylin and Eosin x 100, normal male Wistar rats after feeding CE (A) and distilled water (B) for 14 days, non-remarkable lesions of liver were illustrated.

A. Group 1: Normal rats fed CE at 1 g/kg body weight/day, for 14 days.

B. Group 2: Normal rats fed distilled water.

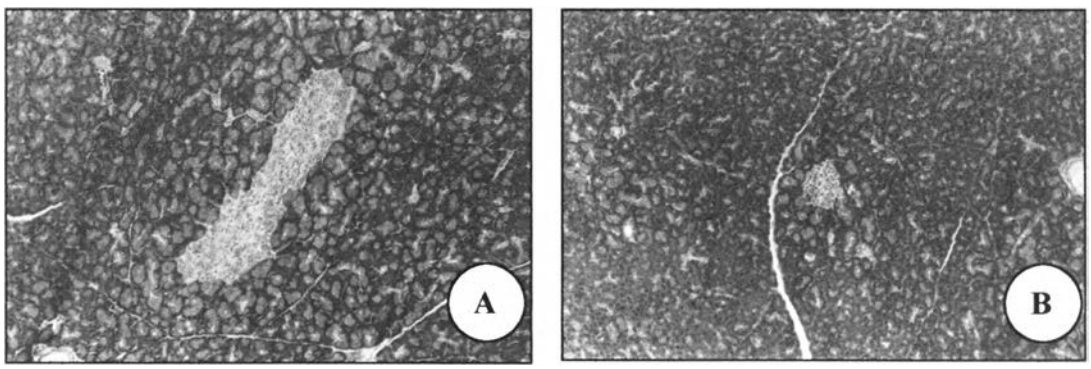

Figure 19. Pancreas, Hematoxylin and Eosin x 100, normal male Wistar rats after feeding CE (A) and distilled water (B) for 14 days, non-remarkable lesions of exocrine pancreas and pancreatic islets were illustrated.

A. Group 1: Normal rats fed CE at 1 g/kg body weight/day, for 14 days.

B. Group 2: Normal rats fed distilled water.

There was no alteration in either serum triglyceride level or serum cholesterol level in CE-treated normal rats (Table 7). There was no difference in biochemical data of serum levels in the treated group from reference values (Appendix I). The result also indicated that CE oral feeding did not affect lipid metabolism in normal rats. Glucose, BUN and creatinine of group 1 showed no significant difference ($p > 0.05$) from group 2 as shown in Table 7. The result reflects that there was no change in β cell, carbohydrate metabolism or kidney damage in rats treated with CE. Hematologic values of both groups showed normal values and were not different (Table 8). As shown in Figures 18 and 19, there were non-remarkable lesions (NRL) in histopathological finding of liver, exocrine pancreas and pancreatic islets, respectively. This suggests that no abnormality occurred in rats treated with 1g/kg body weight/day for 14 days.

2. The hypoglycemic effect of CE in diabetic male Wistar rats.

2.1 Effect of single-oral dose of oral CE on non-fasting blood glucose concentration in diabetic rats.

Effect of oral single-dose of CE on non-fasting blood glucose concentration has been studied. The result showed in Figure 20. Rats in treatment group 1, 2 and 3 were given CE orally at doses 0.10, 0.50 or 1 g/kg body weight, respectively. Rats in standard group were injected insulin 5 IU/kg, subcutaneously, and control group was fed distilled water. Blood glucose concentration was measured every 1 hr for 6 hr. There was no significant difference ($p > 0.05$) in blood glucose levels of treatment group 1 and 2 at 1-3 hr after CE feeding. Treatment group 3 exhibited significant hypoglycemia at 2 ($p < 0.01$) and at 3 ($p < 0.05$) hr after CE feeding and blood glucose level kept lower until reaching a level slightly below 300 mg/dl at 6 hr. Standard group showed distinct hypoglycemia ($p < 0.01$) after 1 hr of insulin injection and remained so throughout 6 hr when compared to the control group.

The result demonstrated that oral CE administration at a single dose of 1 g/kg body weight in diabetic rats could significantly decrease the non-fasting blood glucose concentration. Blood glucose of treatment group 3, standard and control were

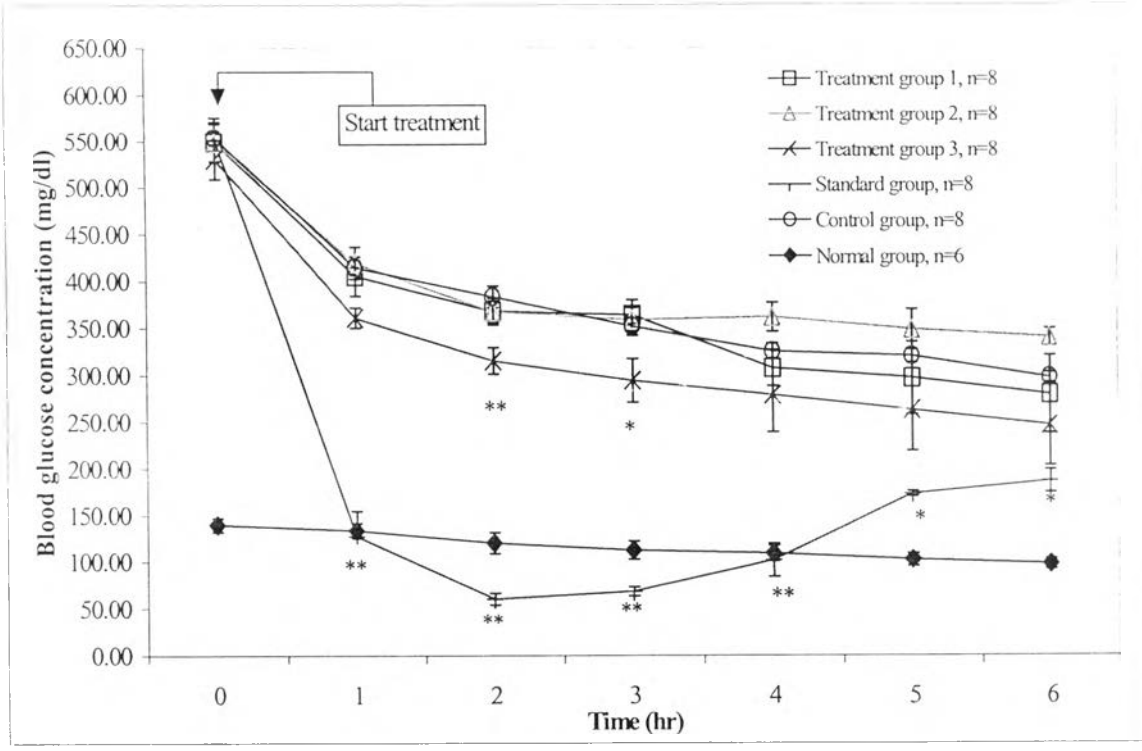

Figure 20. Effect of oral feeding CE on non-fasting blood glucose concentration in streptozotocin-induced diabetic male Wistar rats.

Symbols represents Mean ± S.E.M.

Treatment group 1: Diabetic rats fed single doses of CE at 0.10 g/kg body weight

Treatment group 2: Diabetic rats fed single doses of CE at 0.50 g/kg body weight

Treatment group 3: Diabetic rats fed single doses of CE at 1g/kg body weight.

Standard group: Diabetic rats injected insulin (Humulin®) 5 IU/kg body weight.

Control group: Diabetic rats fed distilled water.

Normal group: Non-diabetic rats.

* Significant at $p < 0.05$, compared to control group.

** Significant at $p < 0.01$, compared to control group.

315.25, 60.20 and 383.20 mg/dl at 2 hr and was 294, 67.90 and 351.64 mg/dl at 3 hr after CE feeding, respectively. Although, hypoglycemic effect of CE showed lower efficiency and shorter duration, but it can suppress significantly the blood glucose level in diabetic rats as compared to the control group.

The mechanism of action CE is not really known. However, the water extract from this plant did decrease blood glucose level in diabetic rats at the highest dose (1g/kg body weight) used in this study. It needs to be clarified whether it does through the elevation of insulin secretion or extra-pancreatic actions influencing glucose metabolism such as, stimulation of glucose uptake by peripheral tissue, correction of insulin resistance and/or inhibition of the endogenous glucose production or activation of the glycogenesis pathway by stimulating glycogen synthetase activity (Jouad *et al.*, 2002). In view of previous observation that a patient of insulin-dependent diabetes type, upon oral administration of CE, her blood glucose decreased from 130 mg/dl normally observed down to 90 mg/dl after insulin injected daily, this suggests that CE might work through a different mechanism than insulin.

2.2 Repeated-oral doses of CE on non-fasting blood glucose in diabetic male Wistar rats.

The effect of repeated CE administration has been demonstrated in Figure 20-23. Rats in treatment group 1, 2 and 3 were given CE orally at 1.00-3.00 p.m. at doses 0.1, 0.5 and 1 g/kg body weight/day, respectively, for 14 days. Rats in standard groups were injected insulin 5 IU/kg body weight/day and in control groups were fed distilled water. On day 0 and day 14, body weight of rats in each group were recorded with no significant difference ($p>0.05$) of body weight (Figure 21), relative body weight gained (Figure 22) was significantly difference ($p<0.05$) in treatment group 2, 3 and standard group when compared with the control group. Relative liver weight of treatment group 3 was significantly difference from control group (Figure 23).

The CE, insulin and distilled water were given in rat once daily for 14 days. Blood was collected on day 7 and 14 at 2.00 p.m., where at least 6 hr after food was taken away and fasting blood glucose concentration were measured. Treatment group

1, 2, 3 and standard showed significant decrease ($p<0.05$) in fasting blood glucose concentration when compared to the control group (Figure 24). On day 7, blood glucose values of treatment group 1, 2, 3, standard and control were 374.33 ± 11.10, 363.83 ± 14.35, 374.04 ± 13.59, 381.32 ± 9.92, 414.51 ± 14.72 mg/dl, on day 14 was 400.83 ± 15.26, 407.37 ± 20.17, 407.33 ± 7.21, 399.54 ± 9.87 and 448.60 ± 13.63 mg/dl, respectively. From this experiment, repeated oral administration have an activity in decreasing blood glucose. Although the exact mechanism of CE is not properly understood, a possible mechanism of action of CE may be increase in glucose utilization in the extrahepatic tissue (Bailey, 1992), reduced hepatic gluconeogenesis (Dong-Hyun *et al.*, 2001; Pushparaj *et al.*, 2000), or increases in expression of insulin receptors in the liver plasma membranes (Pushparaj *et al.*, 2000) or the combination of these.

Serum enzyme levels of AST, ALT, ALP are shown in Table 9. AST values of treatment group 1, 2, 3 and normal rats were significantly lower ($p<0.05$) than the control rats. Oral CE administration in treatment groups at doses 0.5, 1 g/kg body weight or insulin injection 5 IU/kg in standard group once daily for 14 days induced significant lower serum AST level ($p<0.05$) when compared to the control group. AST is an enzyme found primarily in heart, muscle and liver with moderate amounts found in skeletal muscle, kidney and pancreas. Concentration of AST in the blood is normally low to about 49U/L except with cellular damage (Daniels, 2002). So, the result suggested that there was no significant cellular damage in CE-treated animals.

ALT and ALP levels of diabetic control group were not significantly different from that of treatment group 1, 2, 3 and standard group diabetic rats but were significantly different ($p<0.05$) when compared with normal rats. Increases of 1-3 folds of normal level were observed. ALT is normally found in high concentration in liver cells, although smaller amounts are found in cardiac, renal and skeletal tissue. The release of hepatocellular enzyme into the bloodstream occurs with injury or diseases affecting the liver. This release will elevate the serum ALT. Consideration may be made in parallel with AST to differentiate hepatic damage from cardiac damage. AST rises more consistently with cardiac damage than does ALT (Daniels, 2002).

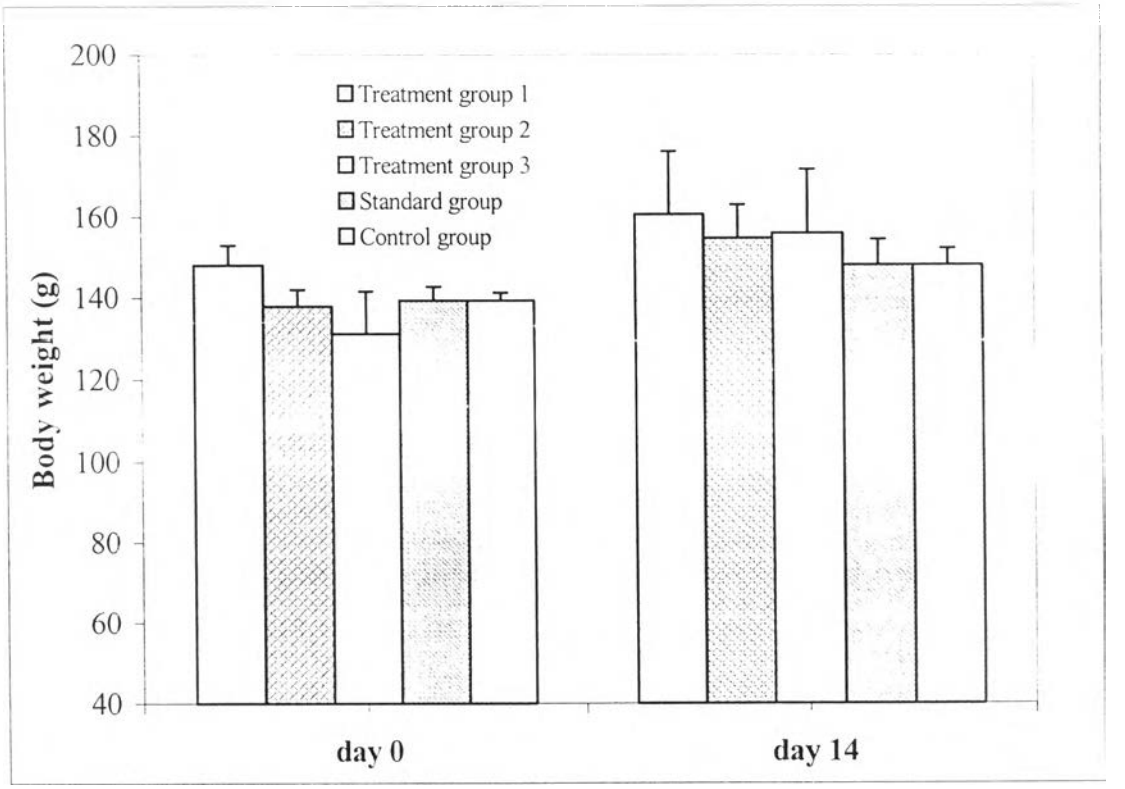

Figure 21. Body weight of rats on day 0 and day 14 after daily oral repeated-doses of CE in diabetic male Wistar rats.

Each bar represents Mean ± S.E.M. Each group of 12 rats.

Treatment group 1: Diabetic rats fed CE at 0.10 g/kg body weight/day, for 14 days.

Treatment group 2: Diabetic rats fed CE at 0.50 g/kg body weight/day, for 14 days.

Treatment group 3: Diabetic rats fed CE at 1 g/kg body weight/day, for 14 days.

Standard group: Diabetic rats injected insulin (Humulin®) 5 IU/kg body weight/day.

Control group: Diabetic rats fed distilled water.

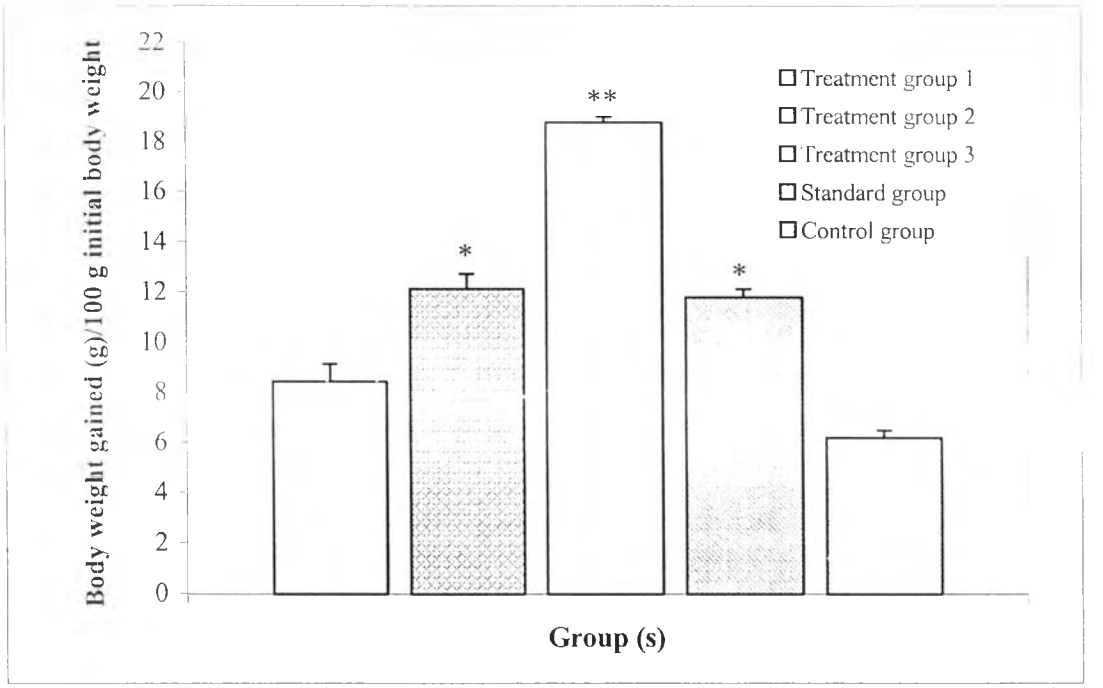

Figure 22. Percentage of relative body weight gained on day 14 after daily oral repeated-doses of CE in diabetic male Wistar rats.

Each bar represents Mean ± S.E.M. Each group of 12 rats.

Treatment group 1: Diabetic rats fed CE at 0.10 g/kg body weight/day, for 14 days.

Treatment group 2: Diabetic rats fed CE at 0.50 g/kg body weight/day, for 14 days.

Treatment group 3: Diabetic rats fed CE at 1 g/kg body weight/day, for 14 days.

Standard group: Diabetic rats injected insulin (Humulin®) 5 IU/kg body weight/day.

Control group: Diabetic rats fed distilled water.

* Significant at $p < 0.05$, compared to control group.

** Significant at $p < 0.01$, compared to control group.

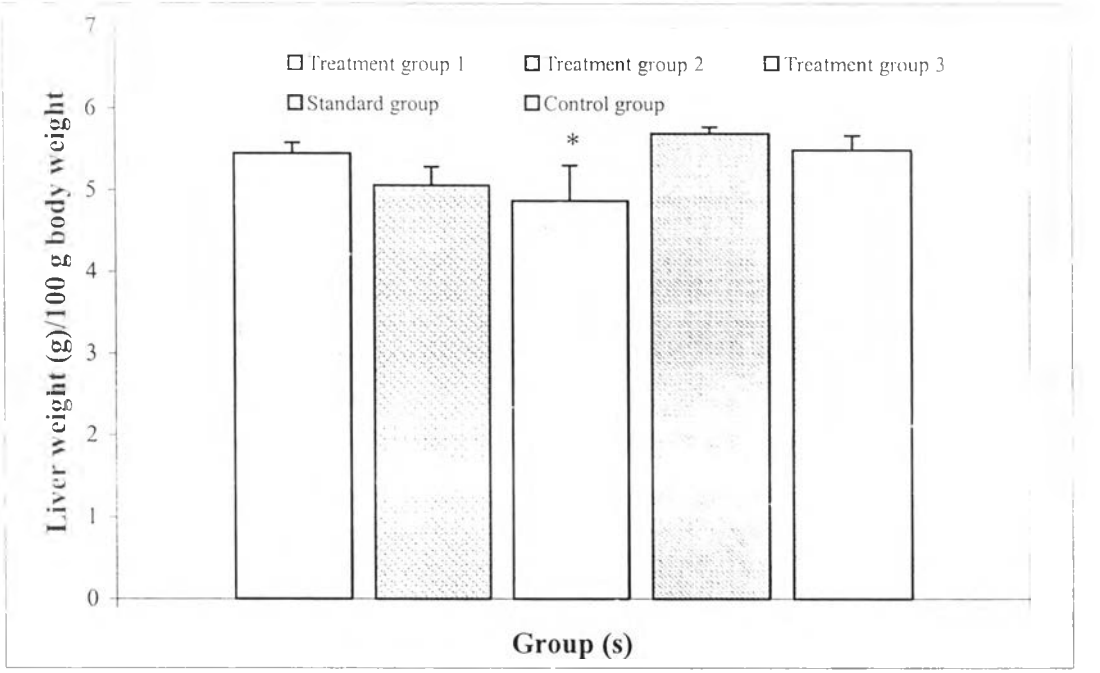

Figure 23. Relative liver weight on day 14 after daily oral repeated-doses of CE in diabetic male Wistar rats.

Each bar represents Mean ± S.E.M. Each group of 12 rats.

Treatment group 1: Diabetic rats fed CE at 0.10 g/kg body weight/day, for 14 days.

Treatment group 2: Diabetic rats fed CE at 0.50 g/kg body weight/day, for 14 days.

Treatment group 3: Diabetic rats fed CE at 1 g/kg body weight/day, for 14 days.

Standard group: Diabetic rats injected insulin (Humulin®) 5 IU/kg body weight/day.

Control group: Diabetic rats fed distilled water.

* Significant at $p < 0.05$, compared to control group.

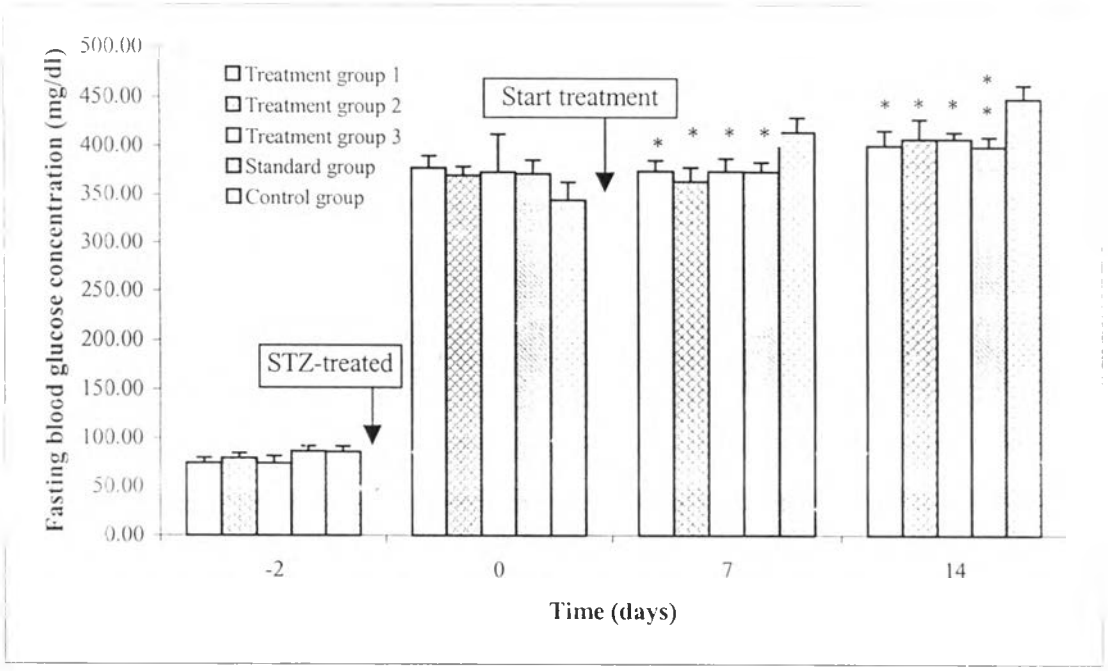

Figure 24. Hypoglycemic effect of CE in streptozotocin-induced diabetic male Wistar rats treated for 7 and 14 days.

Each bar represents Mean ± S.E.M. Each group of 12 rats. STZ = Streptozotocin

Treatment group 1: Diabetic rats fed CE at 0.10 g/kg body weight/day, for 14 days.

Treatment group 2: Diabetic rats fed CE at 0.50 g/kg body weight/day, for 14 days.

Treatment group 3: Diabetic rats fed CE at 1 g/kg body weight/day, for 14 days.

Standard group: Diabetic rats injected insulin (Humulin®) 5 IU/kg body weight/day.

Control group: Diabetic rats fed distilled water.

* Significant at $p < 0.05$, compared to control group.

** Significant at $p < 0.01$, compared to control group.

ALP is a group of enzymes found primarily in the liver, gall bladder, intestinal and bone tissue. The test is done primarily to assist in the diagnosis of hepatic and bone disease (Daniels, 2002; Sodikoff, 1995). From experimental results, ALT and ALP levels of diabetic rats were higher than in normal rats, which may indicate the damage occurred normally in diabetic patients. Etiology of increasing level of ALT and ALP may found in drug-induced toxicity, hepatic cancer or pancreatitis.

Biochemical analysis of serum was demonstrated in Table 10. Cholesterol level of diabetic rats increases about 3 folds higher ($p<0.05$) than normal rats. That of serum cholesterol of treatment group 3, standard and normal rats were significantly low ($p<0.05$) in comparison to control group. This study showed that oral feeding CE at a dose of 1 g/kg body weight/day given to diabetic rats could reduce serum cholesterol level to a comparable level of normal rats. Cholesterol is found in animal fat and is a component of cell membranes. It can be metabolized to bile acids, adrenal steroids and hormones. Cholesterol is combined with protein when being transported in the blood and are termed lipoproteins (Van-Horn, 1996).

Triglyceride value in diabetic rats is significantly higher ($p<0.01$) than that of normal rats (Table 10). This is true in diabetic patients which always have high triglyceride levels (Traub, 1996). All of CE-treated groups showed significantly triglyceride reduction ($p<0.05$) compared to control group. Furthermore, triglyceride levels of CE-treated groups also remained lower than insulin-treated group. Triglycerides are esters of glycerol with fatty acids. They are often transported in combination with protein. The serum triglyceride level in treatment groups the levels were only half or less than half of that of control diabetic rats and lower than that of insulin-injected diabetic rats. It is true for serum triglyceride level. This lower level of both serum lipids should be very beneficial in diabetes, as this would minimize chance of developing atherosclerosis normally found in diabetic patients.

Glucose levels in serum of normal rats were significantly different ($p<0.01$) from diabetic rats. It is clear that the diabetogenic effect of STZ is the direct result of irreversible damage to pancreatic β cell, allowing degranulation and loss of insulin

Table 9. Level of serum enzymes in diabetic male Wistar rats after treatment for 14 days.

Group	N	Body weight (g)	AST (units)	ALT (units)	ALP (units)
Treatment group 1	12	160.56 ± 2.35	257.43 ± 41.86	101.57 ± 16.48	1375.57 ± 336.10
Treatment group 2	12	154.61 ± 3.50	151.33 ± 66.61*	124.25 ± 35.68	1616.87 ± 260.74
Treatment group 3	12	157.42 ± 4.57	88.25 ± 18.01**	73.25 ± 13.77	1011.75 ± 134.19
Standard group	12	150.92 ± 5.14	206.75 ± 46.32*	98.54 ± 13.30	1125.15 ± 83.95
Control group	12	151.42 ± 2.15	388.82 ± 106.20	130.0 ± 30.98	1306.63 ± 140.53
Normal group	6	251.00 ± 3.56	133.5 ± 36.21*	51.33 ± 10.17*	426.83 ± 60.03**

* Significant at $p < 0.05$, compared to control group.

** Significant at $p < 0.01$, compared to control group.

Data are Mean ± S.E.M.

Treatment group 1: Diabetic rats fed CE at 0.10 g/kg body weight/day, for 14 days.

Treatment group 2: Diabetic rats fed CE at 0.50 g/kg body weight/day, for 14 days.

Treatment group 3: Diabetic rats fed CE at 1 g/kg body weight/day, for 14 days.

Standard group: Diabetic rats injected insulin (Humulin®) 5 IU/kg body weight/day.

Control group: Diabetic rats fed distilled water.

Normal group: Non-diabetic rats.

Table 10. Biochemical analysis of serum in diabetic male Wistar rats after treatment for 14 days.

Group	Glucose (mg%)	Cholesterol (mg%)	Triglyceride (mg%)	BUN (mg%)	Creatinine (mg%)
Treatment group 1	400.83 ± 15.26*	103.00 ± 8.85	287.43 ± 65.16*	40.43 ± 1.87	0.48 ± 0.02
Treatment group 2	407.37 ± 20.17*	101.25 ± 8.92	237.63 ± 56.51*	36.25 ± 1.83	0.48 ± 0.02
Treatment group 3	407.33 ± 7.21*	71.00 ± 9.53*	221.25 ± 41.76*	44.95 ± 6.71	1.13 ± 0.38*
Standard group	399.54 ± 9.87*	90.23 ± 5.51*	388.10 ± 61.85	39.74 ± 1.87	0.58 ± 0.06
Control group	448.60 ± 13.63	225.96 ± 10.44	585.63 ± 166.78	43.43 ± 1.56	0.65 ± 0.13
Normal group	121.45 ± 10.33**	67.33 ± 4.66*	119.00 ± 20.49**	22.33 ± 1.52**	0.52 ± 0.03

* Significant at $p < 0.05$, compared to control group.

** Significant at $p < 0.01$, compared to control group.

Data are Mean ± S.E.M.

Treatment group 1: Diabetic rats fed CE at 0.10 g/kg body weight/day, for 14 days.

Treatment group 2: Diabetic rats fed CE at 0.50 g/kg body weight/day, for 14 days.

Treatment group 3: Diabetic rats fed CE at 1 g/kg body weight/day, for 14 days.

Standard group: Diabetic rats injected insulin (Humulin®) 5 IU/kg body weight/day.

Control group: Diabetic rats fed distilled water.

Normal group: Non-diabetic rats.

Table 11. Hematological parameters of diabetic male Wistar rats after treatment for 14 days.

Group	RBC (Cell x 10^6 / mm^3)	Hb (%)	Hct (%)	WBC (Cell x 10^3 / mm^3)	Neu (%)	Band (%)	Eos (%)	Baso (%)	Lymp (%)	Mono (%)
Treatment group 1	7.14 ± 0.38	14.00 ± 0.63	47.50 ± 1.67	4.70 ± 1.37	34.75 ± 10.27*	---	---	---	65.25 ± 10.27*	---
Treatment group 2	5.71 ± 0.75	13.01 ± 0.75	45.55 ± 3.48	13.32 ± 7.97	19.37 ± 5.12	---	0-1	---	80.13 ± 5.13	---
Treatment group 3	6.85 ± 0.23	12.89 ± 0.65	46.75 ± 2.57	5.56 ± 0.98	25.42 ± 7.40	---	0-1	---	90.33 ± 5.24	---
Standard group	5.78 ± 0.85	12.70 ± 1.33	44.50 ± 4.86	7.35 ± 1.30	14.67 ± 4.49	---	---	---	85.33 ± 4.48	---
Control group	6.51 ± 0.12	14.46 ± 0.59	48.73 ± 3.94	6.56 ± 0.13	14.40 ± 6.82	---	---	---	85.60 ± 6.82	---
Normal group	6.10 ± 0.33	13.33 ± 0.69	48.88 ± 3.38	5.73 ± 1.43	6.80 ± 1.46	---	0-1	---	92.80 ± 1.32	---

* Significant at $p < 0.05$, compared to control group.

Data are Mean ± S.E.M.

Treatment group 1: Diabetic rats fed CE at 0.10 g/kg body weight/day, for 14 days.

Treatment group 2: Diabetic rats fed CE at 0.50 g/kg body weight/day, for 14 days.

Treatment group 3: Diabetic rats fed CE at 1 g/kg body weight/day, for 14 days.

Standard group: Diabetic rats injected insulin (Humulin®) 5 IU/kg body weight/day.

Control group: Diabetic rats fed distilled water.

Normal group: Non-diabetic rats.

secretion (Arison and Fendale, 1967; Arison *et al.*, 1967). Bonnevie-Neilsen *et al.* (1981) demonstrated that after STZ treatment, a major loss of β cells and a concomitant decrease of insulin release capacity precedes hyperglycemia, as well as the peak incidence of insulitis. However, the result in CE treated in diabetic rats showed significant lower blood glucose level ($p<0.05$) than control group. This result showed that CE could suppress blood glucose level in diabetic rats. This signifies the fact that CE might play a corrective role in liver function either by reducing the blood glucose level or by some other mechanisms, which in turn reduced the level of triglyceride and cholesterol in blood serum of diabetic animals (Dong-Hyun *et al.*, 2001).

Serum BUN of CE-treated diabetic rats, standard and control groups were not different ($p>0.05$). However, they were significantly different ($p<0.01$) from normal rats. BUN, the nitrogen component of urea, is the end product of protein metabolism. It is formed by the liver from ammonia and excreted by the kidney. BUN reflects protein intake, the liver's ability to metabolize and the renal excretory ability. BUN exists in a normal ratio with serum creatinine and they often rise together in pathological conditions of the renal system (Daniels, 2002). This result obtained might suggest certain degree of kidney damage in diabetic rat, compared to the normal rats.

Creatinine values of group 1, 2, standard and normal rats were not significantly different ($p>0.05$) from that of control group. But group 3 was significantly higher ($p<0.05$) when compared to the control group. Creatinine is the waste product of muscle metabolism and is excreted by the kidney. Its level is a reflection of the body muscle mass. Creatinine is not dramatically affected by fluid balance, nutritional status or liver function, as in the blood urea nitrogen (BUN) test. The serum creatinine levels remains constant and normal in the presence of normal renal function (Daniels, 2002; CWS, 2002). The results of creatinine and BUN could indicate some impairment of kidney function.

Hematologic data are expressed in Table 11. CBC of all 3 treatment groups were not significantly different, except for neutrophils and lymphocytes percentage

which are significantly different ($p<0.05$) from that of control group. Neutrophils are the main defender of the body against infection and antigen. Lymphocytes are the major cellular component of immunity in the body (Sodikoff, 1995). Depressed levels of lymphocytes and increased levels of neutrophils suggests an exhausted immune system as well as a possible body defense mechanism against foreign substance (CWS, 2002).

Non-remarkable lesions in histopathological finding of liver, pancreas and pancreatic islets in all 3 treatment groups, standard, control and normal groups were observed (Figure 25-26), except for a few rats showed some lesions such as mild-periacinar degeneration, mild mid-zonal degeneration, focal necrosis, focal coagulative necrosis, in liver or focal necrosis, mild vascular degeneration, focal hemorrhage in exocrine pancreas or pancreatic islet.

4. The acute toxicity and LD_{50} determination tested in normal male Wistar rats.

The result of acute toxicity test in normal male Wistar rats demonstrated that CE did not induce any sign of acute toxicity within 5 days. Noticeable changes in diet and water consumption was not observed, except on the day of treatment less food intake in treated rats was remarked. CE-treated group had lower body weight than that of control group (Figure 27), however, in Figure 28 showed relative body weight gained was significant difference ($p<0.05$). No clinical signs were observed in treatment group 1 and 4 except for treatment group 2 and 3, i.e. signs of physiological diarrhea were found on day 0 and 1. Relative liver weight of the CE-treated rats showed no significant difference ($p>0.05$) from the control group (Figure 29).

The assessment of pathological changes and toxic effect are also characterized by high levels of serum biochemical parameters as well as some specific enzymes in serum, such as AST, ALT and ALP (Fauci *et al.*, 1998). Data on serum enzyme AST, ALT, ALP are shown in Table 12. Level of AST, ALT and ALP enzymes among treatment group 1, 2 and 3 were not significantly different ($p>0.05$) compared to the control group, which suggests that pathological change of liver function had not occurred (Fauci *et al.*, 1998).

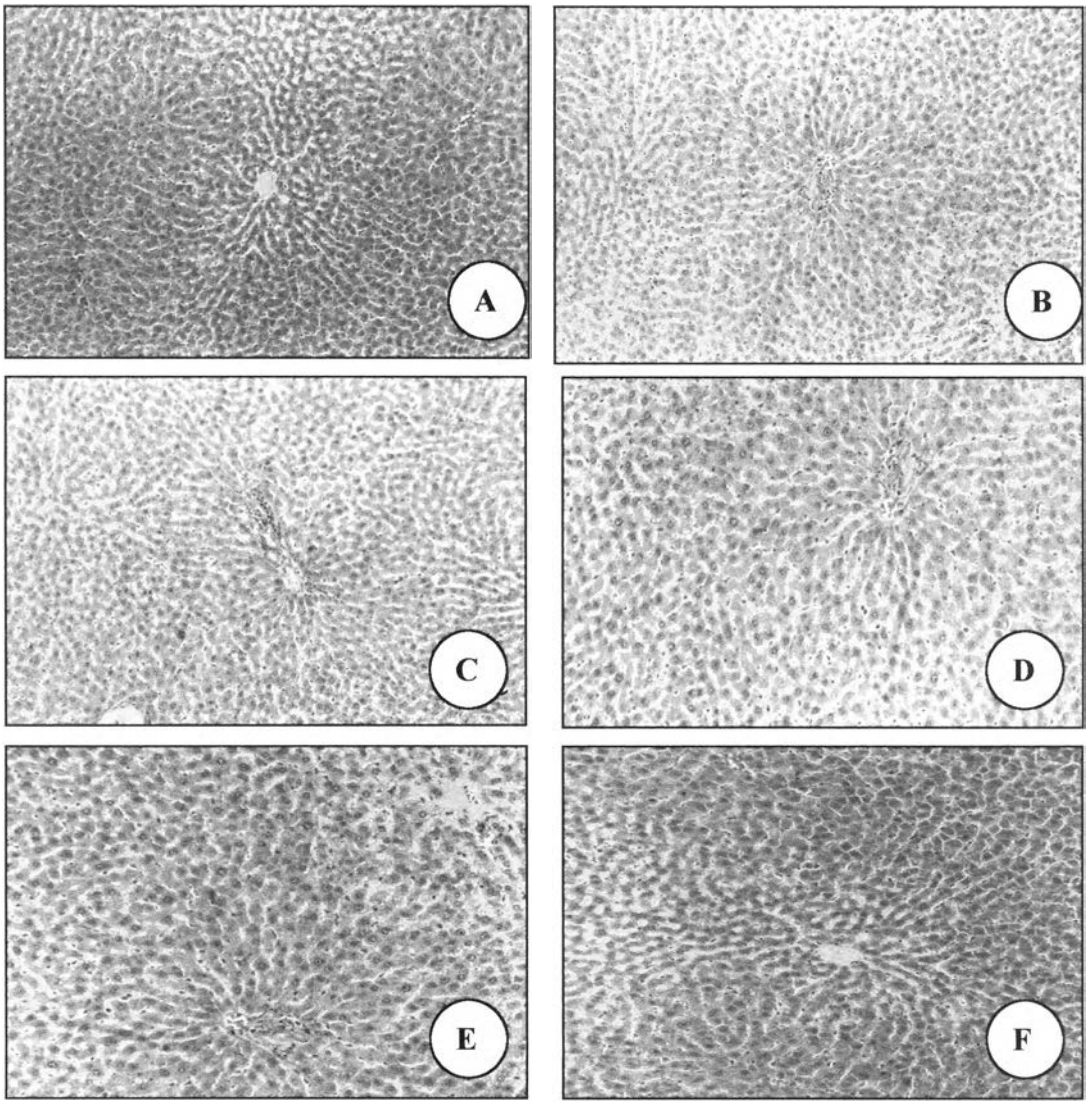

Figure 25. Rat liver after treatment for 14 days. The liver cell cords converge toward the central vein. Portal areas are marked with bile ducts, artery and vein. Hematoxylin and Eosin stain x 100, non-remarkable lesions of liver were illustrated.

A. Treatment group 1: Diabetic rats fed CE at 0.10 g/kg body weight/day, for 14 days.

B. Treatment group 2: Diabetic rats fed CE at 0.50 g/kg body weight/day, for 14 days.

C. Treatment group 3: Diabetic rats fed CE at 1 g/kg body weight/day, for 14 days.

D. Standard group: Diabetic rats injected insulin (Humulin®) 5 IU/kg body weight/day.

E. Control group: Diabetic rats fed distilled water.

F. Normal group: Non-diabetic rats.

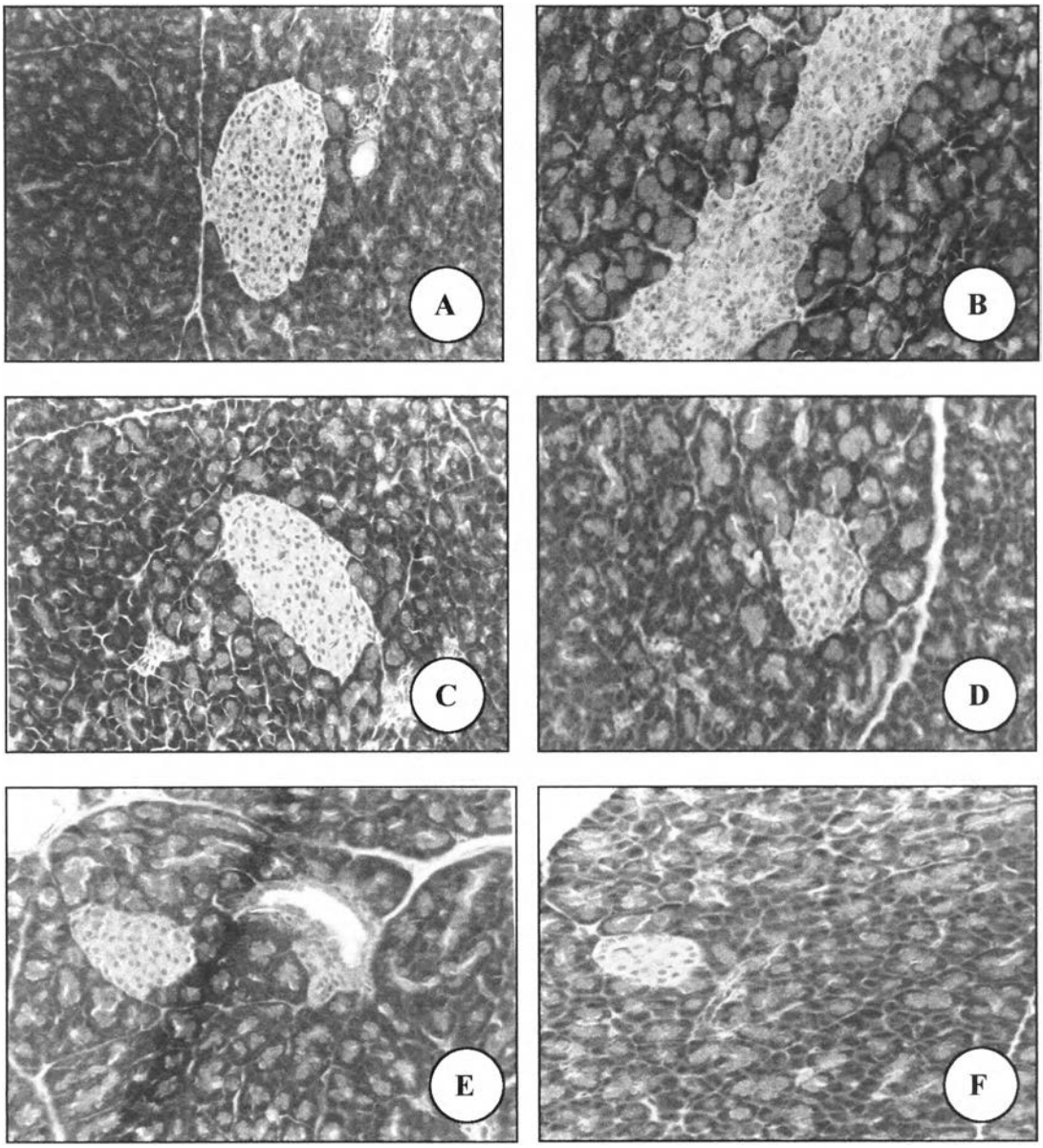

Figure 26. Rat pancreas after treatment for 14 days. The light-staining apical portion of the pancreatic exocrine cells contains zymogen granules, the base dark-staining ergastoplasm. Note the presence of pancreatic islets. Hematoxylin and Eosin stain x 200, non-remarkable lesions of exocrine pancreas and pancreatic islets were illustrated.

A. Treatment group 1: Diabetic rats fed CE at 0.10 g/kg body weight/day, for 14 days.

B. Treatment group 2: Diabetic rats fed CE at 0.50 g/kg body weight/day, for 14 days.

C. Treatment group 3: Diabetic rats fed CE at 1 g/kg body weight/day, for 14 days.

D. Standard group: Diabetic rats injected Insulin-Humulin® 5 IU/kg body weight/day.

E. Control group: Diabetic rats fed distilled water.

F. Normal group: Non-diabetic rats.

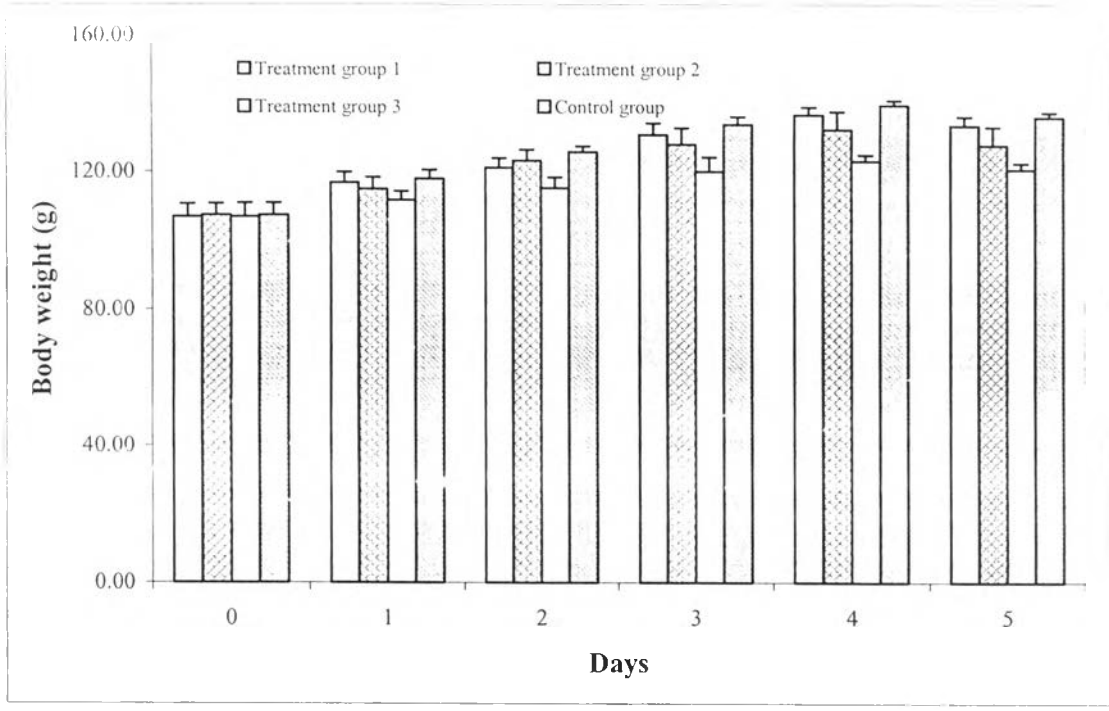

Figure 27. Body weight of rats on day 1–5 post-treatment with CE at a high oral single-dose.

Each bars are Mean ± S.E.M. Each group of 10 rats.

Treatment group 1: Rats fed single doses of CE at 5 g/kg body weight, on day 0.

Treatment group 2: Rats fed single doses of CE at 10 g/kg body weight, on day 0.

Treatment group 3: Rats fed single doses of CE at 20 g/kg body weight, on day 0.

Control group: Rats fed distilled water.

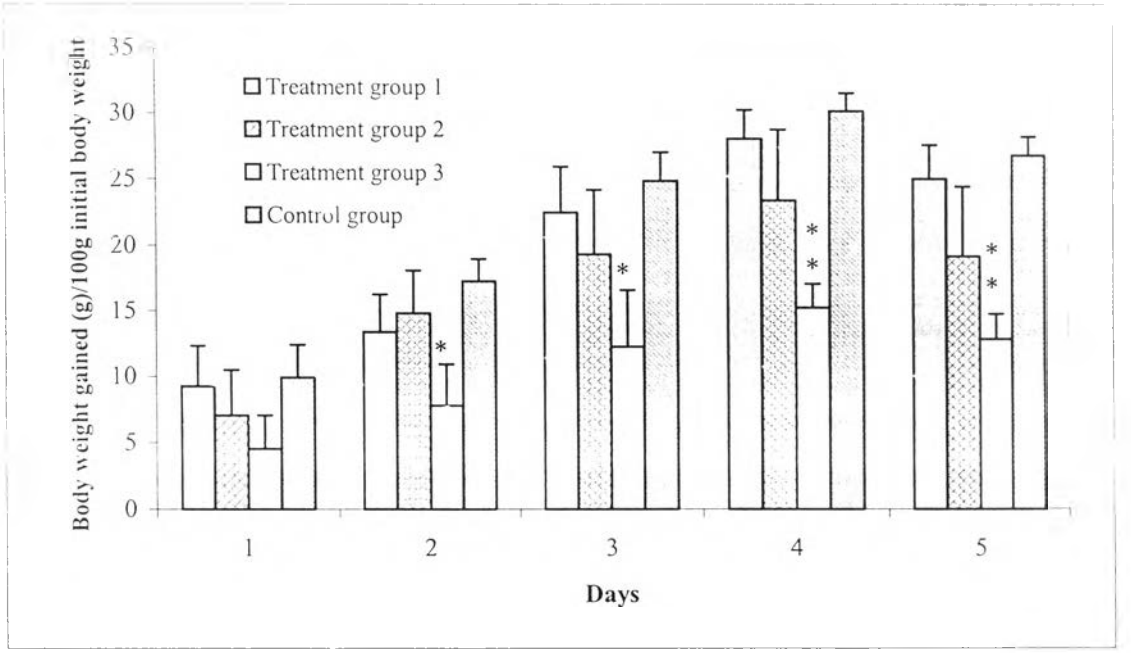

Figure 28. Percentage of relative body weight gained on day 5 post-treatment with CE at a high oral single-dose.

Each bars are Mean ± S.E.M. Each group of 10 rats.

Treatment group 1: Rats fed single doses of CE at 5 g/kg body weight, on day 0.

Treatment group 2: Rats fed single doses of CE at 10 g/kg body weight, on day 0.

Treatment group 3: Rats fed single doses of CE at 20 g/kg body weight, on day 0.

Control group: Rats fed distilled water.

* Significant at $p < 0.05$, compared to control group.

** Significant at $p < 0.01$, compared to control group.

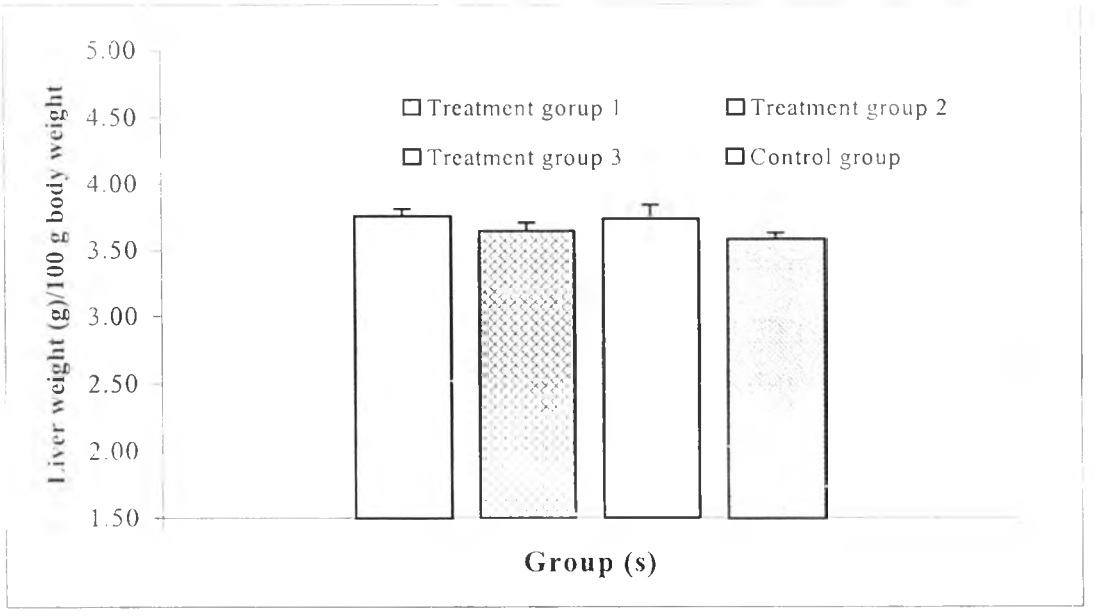

Figure 29. Relative liver weight in rats on day 5 post-treatment with CE at a high oral single-dose.

Each bars are Mean ± S.E.M. Each group of 10 rats.

Treatment group 1: Rats fed single doses of CE at 5 g/kg body weight, on day 0.

Treatment group 2: Rats fed single doses of CE at 10 g/kg body weight, on day 0.

Treatment group 3: Rats fed single doses of CE at 20 g/kg body weight, on day 0.

Control group: Rats fed distilled water.

Biochemical analysis of cholesterol, triglyceride, glucose, BUN and creatinine are shown in Table 13. Cholesterol, triglyceride and glucose concentration of treatment group 1, 2 and 3 were not significant different ($p>0.05$) from control group. The result expressed that treatment did not affect the impairment of the cardiovascular system. In addition, no significant difference of serum BUN and creatinine concentration between treated and control groups were observed. The result obtained seem to indicate that in treated animals did not produce any kidney damage (Wildmann, 1984).

Hematology parameters are illustrated in Table 14. RBC, Hb, Hct values among all groups, i.e.; between treatment group 1, 2, 3 and control were not significantly different ($p>0.05$). WBC, neutrophils, basophils, eosinophils, bands and monocytes were not significantly different from the control group, except for the percentage of lymphocyte of treatment group 3 was significantly lower ($p<0.05$) when compared with the control group. Decreasing level of lymphocytes may indicate an exhausted immune system because neutrophils value are not elevated, because if the neutrophil level is also high may indicate an active infection (CWS, 2002).

Non remarkable lesions in histopathological finding of liver, exocrine pancreas and pancreatic islets were detected after a high oral single-dose of CE administration (Figure 30-31). Mortality was not observed at 24 hr after treatment and throughout the following 5 days post-treatment. CE seems to have minimum adverse effect, with an LD_{50} of greater than 20 g/kg body weight. This results suggest that there may be slight physiological diarrhea effect in rats given CE at dose 10 and 20 g/kg body weight, but no clinical sign in rats at dose 5 g/kg body weight of CE. Thus, lower dose of CE as 5 g/kg body weight was recommended to use in rats.

Table 12. Level of serum enzymes in normal male Wistar rats on day 5 after feeding a high oral single-dose of CE or distilled water.

Group	N	Dose	Body weight (g)	AST (units)	ALT (units)	ALP (units)
Treatment group 1	10	CE 5 g/kg	133.65 ± 2.58	110.20 ± 16.82	46.40 ± 3.19	299.30 ± 43.38
Treatment group 2	10	CE 10 g/kg	127.93 ± 5.26	154.37 ± 40.10	54.10 ± 4.34	269.63 ± 27.16
Treatment group 3	10	CE 20 g/kg	120.64 ± 1.94	102.25 ± 9.33	40.11 ± 5.27	363.55 ± 44.96
Control group	10	Distilled water 3 ml/kg	136.10 ± 1.43	102.40 ± 10.61	43.50 ± 3.90	366.20 ± 20.94

Data are Mean ± S.E.M.

Table 13. Biochemical analysis of serum in normal male Wistar rats on day 5 after feeding a high oral single-dose of CE or distilled water.

Group	Glucose (mg%)	Cholesterol (mg%)	Triglyceride (mg%)	BUN (mg%)	Creatinine (mg%)
Treatment group 1	132.30 ± 9.01	87.10 ± 2.86	67.50 ± 6.74	23.30 ± 2.13	0.44 ± 0.02
Treatment group 2	113.18 ± 4.14	84.27 ± 3.88	80.27 ± 7.69	23.00 ± 2.02	0.47 ± 0.03
Treatment group 3	123.44 ± 7.86	75.00 ± 3.82	58.33 ± 6.60	24.00 ± 2.22	0.44 ± 0.02
Control group	125.40 ± 6.16	78.80 ± 3.59	70.00 ± 8.76	20.80 ± 1.52	0.43 ± 0.02

* Significant at $p < 0.05$, compared to control group.

Data are Mean ± S.E.M. Each group of 10 rats.

Treatment group 1: Rats fed single doses of CE at 5 g/kg body weight, on day 0.

Treatment group 2: Rats fed single doses of CE at 10 g/kg body weight, on day 0.

Treatment group 3: Rats fed single doses of CE at 20 g/kg body weight, on day 0.

Control group: Rats fed distilled water 3ml/kg.

Table 14. Hematological parameters of normal male Wistar rats on day 5 after feeding a high oral single-dose of CE or distilled water.

Group	RBC (Cell x 10^6 / mm^3)	Hb (%)	Hct (%)	WBC (Cell x 10^3 / mm^3)	Neu (%)	Band (%)	Eos (%)	Baso (%)	Lymp (%)	Mono (%)
Treatment group 1	5.05 ± 0.24	11.46 ± 0.39	34.33 ± 2.19	7.82 ± 1.41	7.77 ± 1.73	---	0-3	---	90.88 ± 1.77	0 - 1
Treatment group 2	5.20 ± 0.29	10.31 ± 0.81	37.98 ± 3.88	5.55 ± 1.17	6.57 ± 1.57	---	0-2	---	92.14 ± 1.80	0 - 1
Treatment group 3	5.06 ± 0.28	10.77 ± 0.65	35.38 ± 2.05	4.65 ± 0.63	8.25 ± 2.33	---	0-3	---	79.87 ± 9.31*	0 - 1
Control group	5.62 ± 0.35	10.66 ± 1.02	34.83 ± 3.13	6.40 ± 0.98	5.44 ± 0.88	---	0-3	---	92.55 ± 1.21	0 - 1

* Significant at $p < 0.05$, compared to control group.

Data are Mean ± S.E.M. Each group of 10 rats.

Treatment group 1: Rats fed single doses of CE at 5 g/kg body weight, on day 0.

Treatment group 2: Rats fed single doses of CE at 10 g/kg body weight, on day 0.

Treatment group 3: Rats fed single doses of CE at 20 g/kg body weight, on day 0.

Control group: Rats fed distilled water.

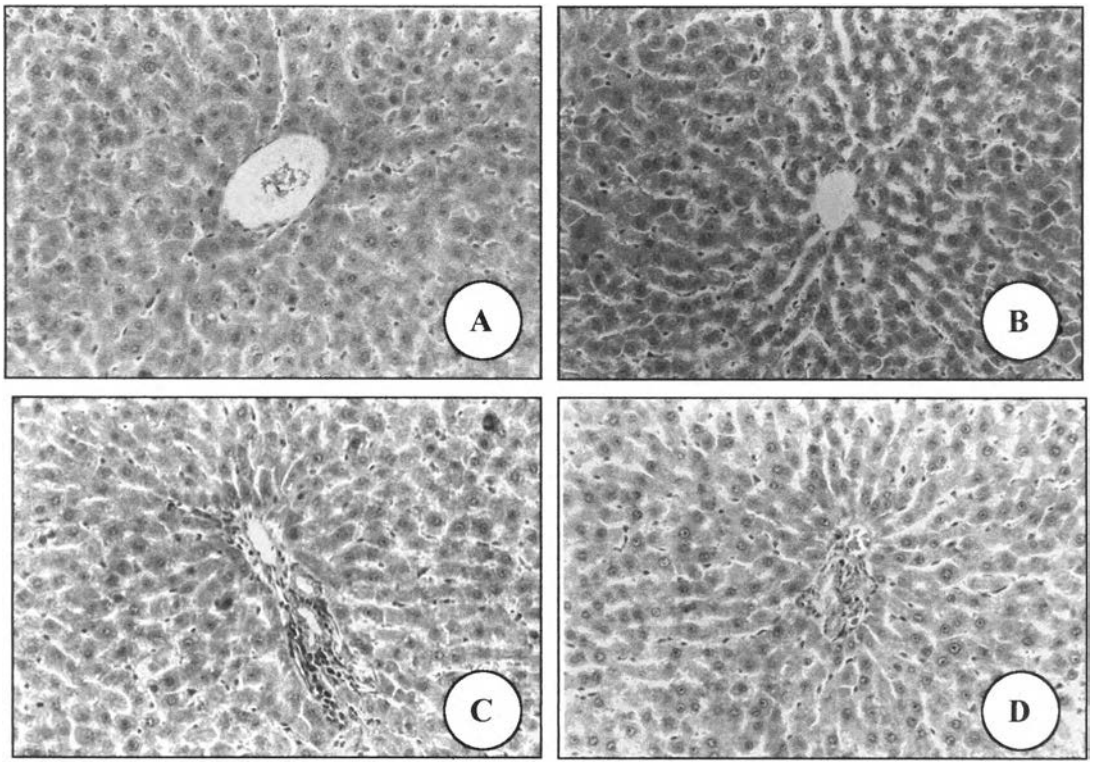

Figure 30. Rat liver after feeding a high oral single-dose of CE or distilled water. Hematoxylin and Eosin stain x 200, non-remarkable lesions of liver were illustrated.

A. Treatment group 1: Rats fed single doses of CE at 5 g/kg body weight, on day 0.

B. Treatment group 2: Rats fed single doses of CE at 10 g/kg body weight, on day 0.

C. Treatment group 3: Rats fed single doses of CE at 20 g/kg body weight, on day 0.

D. Control group: Rats fed distilled water.

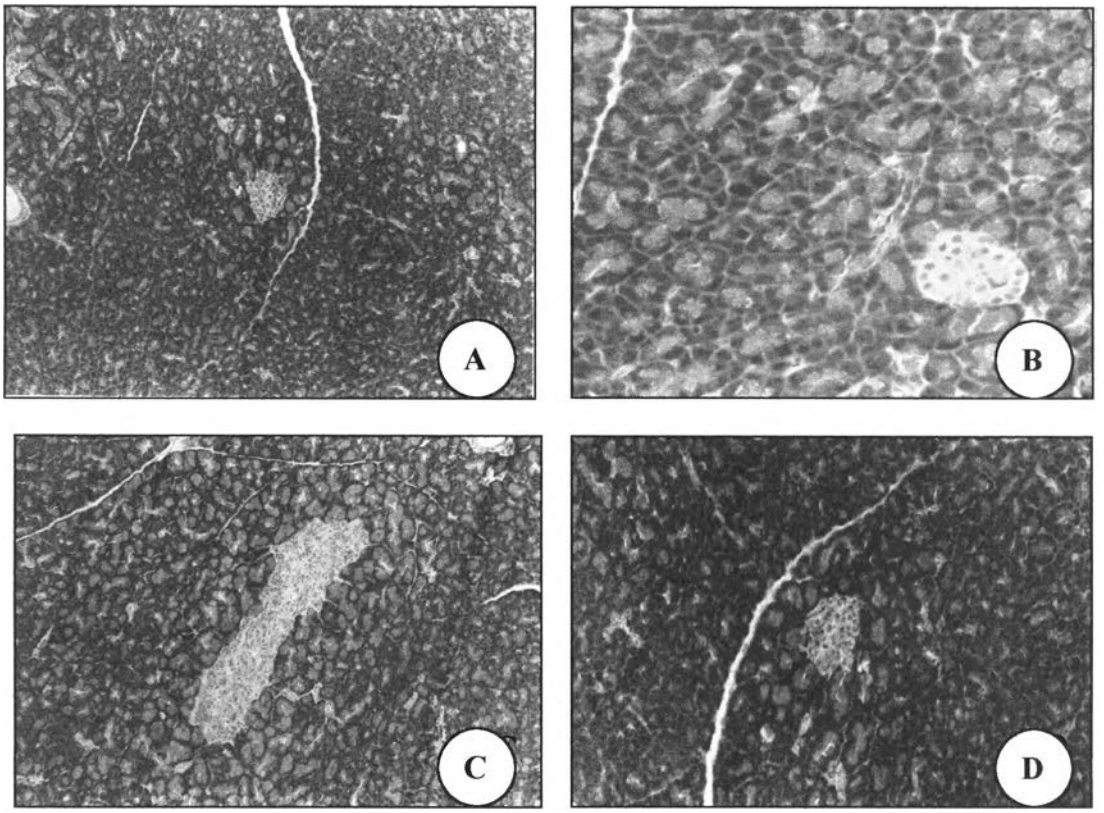

Figure 31. Rat pancreas after feeding a high oral single-dose of CE or distilled water. Hematoxylin and Eosin stain x 100, non-remarkable lesions of exocrine pancreas and pancreatic islets were illustrated.

A. Treatment group 1: Rats fed single doses of CE at 5 g/kg body weight, on day 0.

B. Treatment group 2: Rats fed single doses of CE at 10 g/kg body weight, on day 0.

C. Treatment group 3: Rats fed single doses of CE at 20 g/kg body weight, on day 0.

D. Control group: Rats fed distilled water.

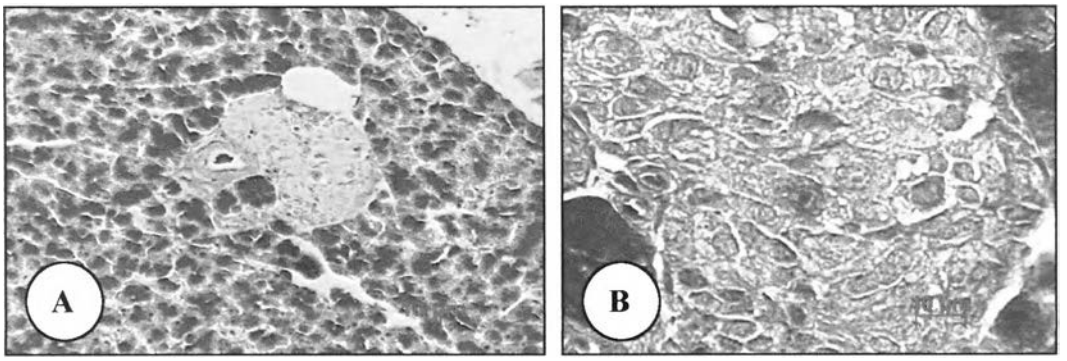

Figure 32. Pancreatic islets, rat β cells. The granules of these polyangular β cells are stained blue with Gomori's chrome method (Ax200), (Bx400). Positive staining of blue granules in cytoplasmic β cell indicate an ability of pancreatic islets to produce insulin.