Protective effect of crude *Curcuma longa* and its methanolic extract in alloxanized rabbits

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Abstract: *Curcuma longa* (*C. longa*) is commonly found in different areas of Pakistan. It has been locally utilized as a traditional medicine. The aim of this study was to evaluate the antidiabetic, hepatoprotective and total antioxidant effect of the crude drug and its methanolic extract in rabbits. Diabetes was induced with alloxan (180mg/kg). Two major groups were designed, curative and protective groups. In curative group the crude drug and its methanolic extract was orally administered to the diabetic animals and acute study was performed. On the other hand in protective group the crude drug and its methanolic extract were administered for eight days prior to the diabetes induction. Results indicated that in Curative group the crude and methanolic extract of *C. longa* significantly improved the levels of serum glucose, serum transaminases and antioxidant activity (AOA). In protective group, serum glucose, serum transaminases were not significantly increased by alloxan, in both crude as well as methanolic extract group. This study shows that *C. longa* acts as antidiabetic, hepatoprotective and antioxidant in diabetes especially type 1 diabetes.

Keywords: *Curcoma longa*; Alloxan; Antidiabetic; Hepatoprotective; Antioxidant

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by increased blood sugar, polyuria and polydipsia. Diabetes mellitus causes immune destruction of beta cells and causes redox imbalance inside the cells, especially in the liver and kidney. The destruction of the cells also leads to decreased antioxidant defense mechanism and increased free radical production. In diabetes mellitus free radical production increases due to increased oxidative stress and antioxidant activity is decreased. Therefore, increased free radical production could be considered one of the important complications of diabetes mellitus (Meral *et al.,* 2001).

Diabetes can be produced in animals by the drugs alloxan and streptozotocin; the mechanism of action of these two drugs is different, but both result in the production of active oxygen species. It was proposed (Lenzen *et al.,* 1996) that alloxan destroys beta cell function by inhibiting glucokinase activity through oxidation of two thiol groups which are in the glucose binding site of the enzyme. Later (Zhang *et al.,* 2007) proposed that alloxan also affects glucokinase activity in liver, another major site of glucokinase expression. Therefore it was hypothesized that part of diabetogenic effect of alloxan is due to its effect on liver. The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are used routinely for assessing liver function. Production of reactive oxygen species also causes changes in kidney tubular cells, hence disturbing the albumin-globulin ratio in the kidneys (Tierney, McPhee and Papadakis, 2002).

C. longa L., which belongs to the Zingiberaceae family, is an erect perennial herb with thick and fleshy rhizomes and leaves in sheaths. The rhizome is the portion of the plant used medicinally. *C. longa* is valued mainly for its principal coloring pigment, curcumin, which imparts the yellow colour to *C. longa*, besides other nutritive constituents like potassium (Chempakam and Parthasarathy, 2008).

C. longa L. possess compounds which are potent inhibitors of inflammation. *C. longa* has antiprotozoal, nematocidal, antibacterial, anti venom, anti HIV and antitumor activity. *C. longa* also possess curcuminoids which have phenolic and enolic structure. These types of structures have the ability to trap radicals and are good antioxidants (Araújo and Leon, 2001).

In the present investigation an attempt has been made to assess the antidiabetic and antihepatotoxic and *in vivo* antioxidant effects of crude and methanolic extract of *C. longa* found locally, in alloxanized rabbits.

MATERIALS AND METHODS

Animals

Male rabbits weighing from 1.5 to 2.5 kg were purchased from the local market. The animals were kept in the animal house of the Faculty of Pharmacy with 12 hr light dark cycle and maintained on 24±2^{o}C normal temperature. They were retained for acclimatization for a period of 1 week before starting the experiment. The rabbits were fed standard fresh green fodder throughout the experiment and water *ad libitum*.

Chemical

Alloxan purchased from Sigma Chemical Co., St. Louis USA was used for the induction of diabetes.

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Preparation of alloxan solution

10% solution of alloxan was prepared freshly in 0.9% sodium chloride solution before injection. 0.9g of sodium chloride was dissolved in 100 ml of water and pH was checked and maintained at pH 5.5 (5-7) by addition of hydrochloric acid or sodium hydroxide. 10% solution of alloxan was prepared and different doses i.e. 70mg/kg, 100mg/kg, 150mg/kg, 180mg/kg and 200mg/kg were injected to five groups of rabbits (n=5). 180 mg/kg was selected for the study as the death rate was low and stable glucose level (200-300mg/dl) was obtained within eight days.

Induction of diabetes

The rabbits were fasted for 12 hr prior to the induction of diabetes mellitus. Blood was collected for zero hour determination of serum glucose and serum transaminases. Rabbits were injected intraperitoneally with 180 mg/kg body weight (b.w.) of freshly prepared 10% alloxan monohydrate (Sigma Chemical Co., St Louis USA) dissolved in isotonic NaCl to induce Diabetes Mellitus at the start of the experiment. Diabetes was developed and stabilized over a period of eight days. The rabbits with fasting glucose range of 200 to 350 mg/dl were considered diabetic and included in the study.

Plant material

Crude *C. longa* Linn. (Zingeberaceae) rhizomes were collected from Kasur district, Punjab, Pakistan. The species was identified by Department of Botany, Government College, Lahore. The rhizomes were peeled, cut into small pieces and shade dried. Crude drug was powdered. In order to obtain content uniformity the crude powder was passed through sieve of 42 mesh size. The crude powder was then preserved in amber colored air-tight glass jars and was placed in refrigerator.

Preparation of crude C. longa suspension

Fresh *C. longa* suspension was freshly prepared daily. Acacia was used with *C. longa* in ratio of 1:4 as suspending agent. A small amount of *C. longa* was triturated with acacia in a pestle and mortar using distilled water as a vehicle. *C. longa* and acacia were triturated until a smooth paste was formed then water was added to make up the volume. *C. longa* suspension was given to rabbits orally using a soft tube.

Preparation of methanolic extract of C. longa

100g of finely powdered crude *C. longa* was soaked in 500ml of methanol for about 15 days. The methanolic extract was stirred at room temperature with electric stirrer for one hour. The extract was filtered and evaporated by using rotary evaporator at temperature less than 40°C. The residue obtained was orange-red colored sticky gummy material because *C. longa* contains curcuminoids mainly curcumin which is an oily residue. The yield was 7.2%. The methanolic extract suspension was freshly prepared daily with acacia for experimental purpose.

Analytical methods

Serum glucose and serum transaminases were determined by using the kit supplied by Randox Lab., U.K. The total antioxidant activity *in vivo* was measured in serum by using method of Koracevic *et al.* (2001). In this method a standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by a fenton type reaction, leading to the formation of hydroxyl radicals (OH). These reactive oxygen species degrade benzoate, resulting in the release of TBARS. Antioxidants from the added sample cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of color development defined as the antioxidant activity.

Experimental design and treatment schedule

The animals were divided into 6 groups containing 6 rabbits in each group. Initially, all the parameters of the rabbits were checked and only healthy rabbits were selected for the study. The experimental groups were as follows:

Curative group

Group 1 Control Diabetic Group (CDG): Alloxan 180mg/kg was injected intraperitoneally and then the rabbits were kept on vehicle (2% gum acacia solution) throughout the experiment.

Group 2 Crude drug Treated group (CTG): The effect of crude *C. longa* (2g/kg of b.w) was determined on serum glucose, ALT and AST in alloxan treated diabetic rabbits 4 hrs, 8 hrs and 24 hr after the administration of crude drug.

Group 3 Methanolic extract Treated group of *C. longa* (MTG): The effect of methanolic extract of *C. longa* equivalent to 2g/kg b.w of crude drug powder was determined on the activity of serum glucose, ALT and AST in alloxan treated rabbits 4 hrs, 8 hrs and 24 hrs after the administration of methanolic extract

Protective group

Group 4-Protective Control group (PCG): The animals were kept on vehicle (2% gum acacia solution) initially for 8 days. Alloxan (180mg/kg) was injected on 9th day along with other two groups and observed for next 15 days for the development of diabetes and hepatic damage.

Group 5-Protective Crude drug group (PCD): Crude drug 2g/kg was given for 8 days initially. Then rabbits were injected with alloxan monohydrate (180mg/kg) and observed for next 15 days for the development of diabetes and hepatic damage.

Group 6 Protective Methanolic extract group of *C. longa* (PMG): Methanolic extract equivalent to 2g/kg

of crude drug was given for 8 days initially. Then rabbits were injected with alloxan monohydrate (180mg/kg) and observed for the next 15 days for the development of diabetes and hepatic damage.

STATISTICAL ANALYSIS

All values were expressed as ±SEM and analyzed using Students t test. P values<0.05 were considered significant. P values were obtained from distribution of t probability chart.

RESULTS

Curative group

The effect of crude and methanolic extract of *C. longa* on serum glucose was observed in alloxanized diabetic rabbits and was compared with the group injected only with alloxan (180 mg/kg of b.w). The results are shown in table 1. The mean glucose level in CDG 8 days after alloxan injection was 242.17 mg/dl and at 8 hrs without any drug was 289.5 mg/dl (↑ 165.18%) whereas the mean glucose level in CTG 8 days after alloxan injection was 218 mg/dl and 8hrs after crude drug administration was 138.8 mg/dl (↓ 36.56 %). The mean glucose level in MTG 8 days after alloxan injection was 290 mg/dl and 8 hrs after ingestion of methanolic extract of crude drug was 155mg/dl (↓ 46.58%). When CTG and MTG were compared with the CDG at 8 hrs after drug administration, a significant decrease in glucose level (P<0.005) was observed in both CTG and MTG. fig. 1 shows the comparison between CDG, CTG and MTG at different time intervals. So, the methanolic extract of *C. longa* appeared to have better hypoglycemic effect as compared to crude *C. longa*.

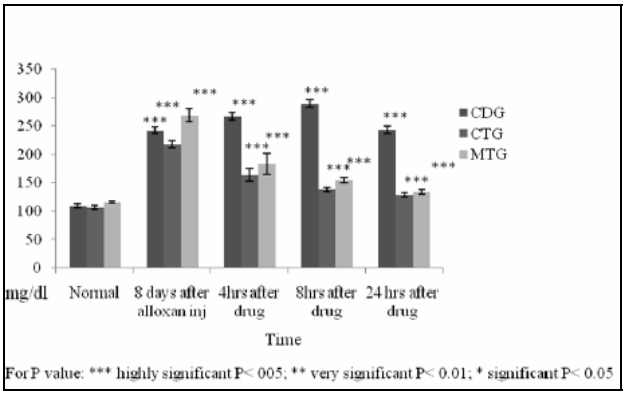

Fig. 1: Comparison of effect of crude and methanolic extract of C.longa with alloxan on serum glucose in diabetic rabbits.

The mean ALT level in diabetic rabbits 8 days after alloxan injection was 69.67 U/l (↑ 492.69%) which shows that there was 5 fold increases in the ALT level in alloxan treated diabetic rabbits. The effect of crude (2g/kg b.w) and methanolic extract of crude drug (equivalent to 2g/kg b.w) can be observed in table 1. The mean ALT level at 4 hrs after drug administration was 70 U/l in CDG (↑ 579.28%). There was significant decrease (p<0.005) in CTG i.e. 39 U/l (↓ 40.53%), when compared with CDG at the same time interval. In MTG the mean ALT level 4 hrs after drug administration was 31.5 U/l (↓ 55%), so significant decrease was observed when compared with CDG (70 U/l at 244 hrs). fig. 2 shows mean ALT levels at different time intervals and it could be observed that MTG reduced ALT more as compared to CTG with the same dose.

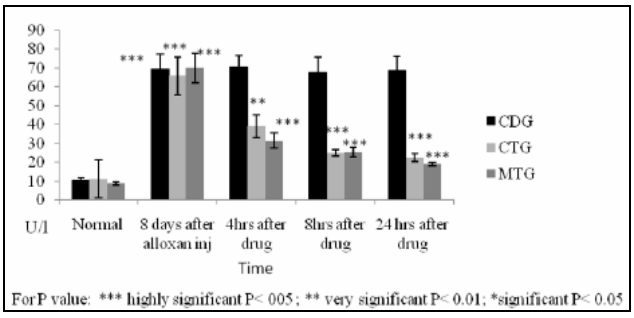

Fig. 2: Comparison of curative effect of crude and methanolic extract of C.longa with alloxan on serum ALT in diabetic rabbits.

AST is another important enzyme for assessing liver function. The normal mean AST level in three groups (i.e. CDG, CTG and MTG) was 10.17, 10.33 and 8.9 U/l. The mean AST level in diabetic rabbits in three groups 8 days after injecting alloxan (180 mg/kg b.w.) was 65.5 (↑ 544.05%), 71.1 (↑ 588.96%) and 69.33 (↑ 678.99) U/l as seen in table 1. It was observed that there was 7 fold increases in AST levels in diabetic rabbits. The mean AST level in alloxan treated rabbits (CDG) 24 hrs after drug administration was 63.33 U/l (↑ 522.71%) and there was significant decrease (P<0.005) in CTG at same time interval i.e. 21.67 U/l (↓ 69.55%). In MTG the mean AST level 24 hrs after drug was 17 U/l (↓ 75.47%). Significant decrease (P<0.005) in mean AST level in MTG was observed when compared with the CDG. Fig. 3 depicts the comparison between CDG, CTG and MTG and significant decrease (P<0.05) in AST level in CTG and MTG could be observed.

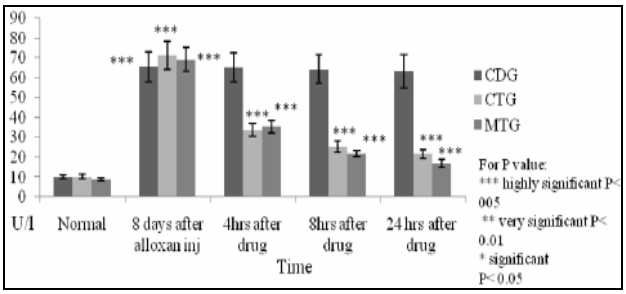

Fig. 3: Comparison of curative effect of crude and methanolic extract of C.longa with alloxan on serum AST in diabetic rabbits.

The mean normal total serum antioxidant level in three groups assessed was 2.1, 2.07 and 2.13 mmol/l shown in table 1. After injection of alloxan the antioxidant level decreased and was 0.713, 0.91 and 0.703 mmol/l in CDG. CTG and MTG presented in table 1. The antioxidant activity decreased upto 0.71 mmol/l (↓ 66.19%) in control group at 8 hrs without any treatment shown in table 1. In CTG the anti oxidant level 8 hrs after ingestion of crude *C. longa* was 1.3 mmol/l (↑42.86%). In MTG group the AOA level at 8 hrs was 1.35 mmol/l (↑92.86%). fig. 4 shows the comparison between CDG, CTG and MTG.

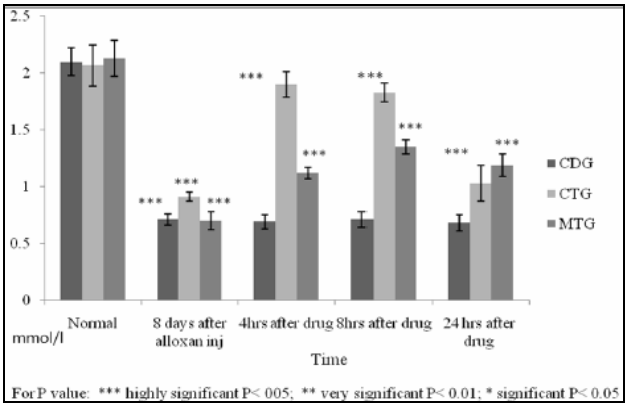

Fig. 4: Comparison of effect of crude and methanolic extract of C.longa with alloxan on serum antioxidant axctivity in diabetic rabbits.

Protective group

The Protective Control Group "PCG" was injected alloxan intraperitoneally (180 mg/kg b.w.) along with the Protective Crude Drug Group "PCD" and Protective Methanolic Extract Group "PMG" after taking the normal glucose and serum transaminases level. The PCD was given crude drug suspension (dose: 2g/kg b.w.) prepared freshly, once daily for 8 days. On the 8th day alloxan (180 mg/kg b.w.) was injected intraperitoneally and the changes in glucose, ALT and AST were observed for the next 15 days. After alloxan injection serum levels of glucose and transaminases were noted on 9th, 17th and 24th days. The third group labeled PMG was given methanolic extract of crude drug suspension prepared freshly for 8 days and treated in same manner as PCD.

The normal glucose level of PCD and PMG observed was 116.3 and 119.5 mg/dl and no significant change in mean glucose levels were observed after 8 days pretreatment with crude drug and its methanolic extract. The mean glucose levels after 8 days pretreatment with crude and methanolic extract of *C. longa* were 115.5 and 118.3 mg/dl shown in table 2. The mean glucose level of PCG was 213 mg/dl (↑ 84.68%) on the 17th day whereas the mean glucose level of PCD and PMG was 166.7 (↑ 43.3%) and 137 (↑ 14.6%) mg/dl respectively as can be seen in table 2. Fig. 5 also shows the comparison of glucose level in PCD and PMG with the PCG.

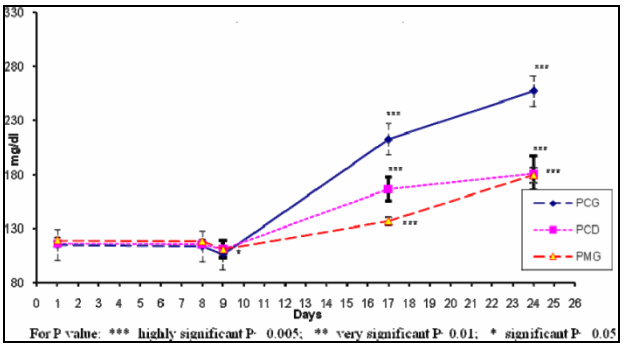

Fig. 5: Study of comparison of protective Effect of crude and methanolic extract of *C. longa* with alloxan on serum glucose in rabbits.

Normal mean ALT levels in PCD and PMG observed were 16.67 and 17 U/l shown in table 2. The mean ALT levels after 8 days pretreatment with crude and methanolic extract of crude drug were 12.16 and 14.5 U/l. No significant changes in mean ALT levels were observed after 8 days pretreatment with crude drug and its methanolic extract. The effect of alloxan (180 mg/kg) and protective effect of crude drug (2 g/kg) and its methanolic extract (equivalent to 2g/kg) on serum ALT level was assessed. The mean ALT level of PCG was 65.42 U/l (↑ 351.17%) on the 17th day whereas the mean ALT of PCD and PMG was 33.8 U/l (↑ 102.9%) and 31.5 U/l (↑ 85.29%) respectively. The comparison among the groups can be observed in fig. 6.

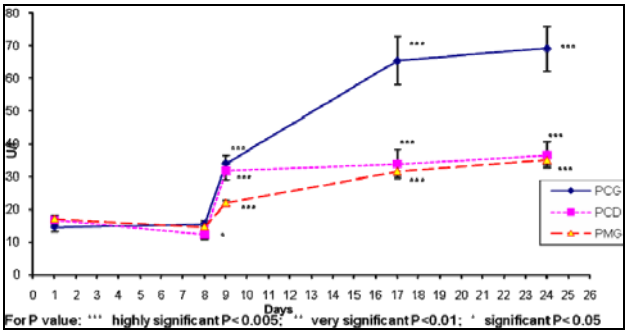

Fig. 6: Study of comparison of protective Effect of crude and methanolic extract of *C. longa* with alloxan on serum ALT in rabbits.

Normal mean AST levels in PCD and PMG observed were 14.67 and 15.83 U/l shown in table 2. The mean AST levels after 8 days pretreatment with crude and methanolic extract of crude drug were 13.17 and 14.3 U/l. No significant changes in mean AST levels were observed after 8 days treatment with crude drug (2g/kg) and its methanolic extract (equivalent to 2g/kg). The mean AST level of PCG was 56.17 U/l (↑ 395.76%) on the 17th day whereas the mean AST levels of PCD and PMG were 32.6 U/l (↑ 102.9%) and 30.7 U/l (↑ 85.29%) respectively. The protective effect of crude *C. longa* and its methanolic extract on AST in PCD and PMG in comparison to PCG can be seen in fig. 7.

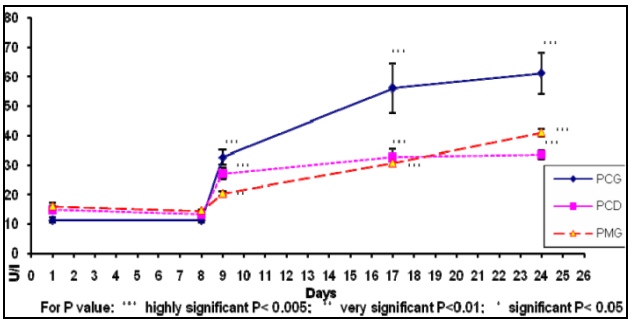

Fig. 7: Study of comparison of protective Effect of *C. longa* and its methanolic extract with alloxan on AST in rabbits.

The total serum antioxidant activity was measured in PCG, PCD and PMG as shown in table 2. The results are compared in fig. 8. After injection of alloxan, the antioxidant activity level in PCG on 8th day decreased up to 0.91 mmol/l (↓ 59.56%) and in PCD was 1.46 mmol/l (↓ 38.66%) and in PMG the change (1.42 mmol/l) was non-significant when compared with normal. In PCG, PCD and PMG the antioxidant level on 17th day was 0.91, 1.46 and 1.35 mmol/l respectively. It can be seen in fig. 8 that the antioxidant level in PMG non-significantly decreased as compared to the PCG.

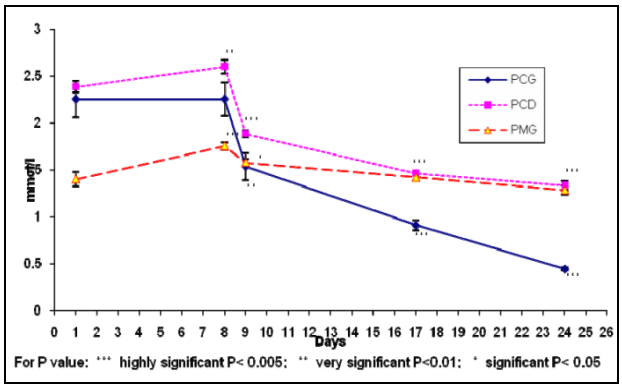

Fig. 8: Study of comparison of protective effect of *C. longa* and its methanolic extract with alloxan on AOA in rabbits.

DISCUSSION

Diabetes mellitus is an irreversible metabolic disorder which is characterized by hyperglycemia due to defects in insulin production and function. In the curative group as shown in table 1 the statistical comparison of alloxan treated is done with normal, crude and methanolic extract of *C. longa*. Highly significant (P<0.005) results were obtained. Alloxan resulted in a significant increase (P<0.005) in serum glucose, ALT and AST enzymatic activity and decreased total antioxidant activity. In groups treated with crude and methanolic extract of *C. longa* the enzymatic values significantly decreased (P<0.005) and total antioxidant activity increased.

In the protective group *C. longa* was first administered for 8 days, alloxan was injected i.p., and then animals were observed for next 15 days. *C. longa* (2g/kg) and its methanolic extract (equivalent to 2g/kg) were administered as a single dose daily for 8 days and before the administration zero hr blood sample was collected. On 8th day alloxan 180mg/kg was injected intraperitoneally once. Sample were taken before alloxan injection and then after 24 hrs, 8 and 15 days. In table no. 2 the protective effect of crude and methanolic extract of *C. longa* is shown on glucose, ALT and AST. Statistical comparison of normal with alloxan treated is shown in table 2 and highly significant (P<0.005) results could be observed.

The mechanism of alloxan has been extensively studied in experimental animal models and is quite well characterized now. Alloxan induces chemical diabetes by destroying the beta cells of the pancreas. The beta cells rapidly uptake the alloxan and cause formation of reactive oxygen species. Alloxan is reduced to dialuric acid in the presence of different reducing agents. It is then reoxidized back to alloxan establishing a redox cycle for the generation of super oxide radicals. As a result of this redox cycle highly reactive hydroxyl radicals are formed. The reactive oxygen species also damage DNA of the pancreatic islets and DNA fragmentation takes place in the beta cells expose to alloxan (Lenzen, 2008; Yali, 2005; Szkudelski, 2001).

Liver is a vital organ responsible for the metabolism of drugs and other substances. Liver functions are carried out by hepatocytes. Damage to the liver cells leads to altered cell membrane permeability and leaking out of tissue content into the blood stream. This in turn leads to increased serum transaminases level (Murugan and Pari, 2007). The changes in serum enzymes are normal in uncomplicated diabetes. The tissue damage caused by the metabolic and circulatory disorders causes' congestion and metabolic disorders which results into liver damage. Increased protein catabolism accompanying gluconeogenesis might be the reason for elevated serum transaminases in diabetic state. *C. longa* and its extract significantly reduced the serum transaminases in both curative and protective groups by reducing hepatocellular damage and suppression of gluconeogenesis. It has also been reported that alloxan destroys beta cell function by inhibiting the glucokinase enzyme activity. Later it was also proved that alloxan inhibits liver glucokinase as well, thus leading to the elevation of serum glucose content that leads to further descrease in insulin secretion. Zhang *et al.* (2007) also demonstrated that liver glucokinase expression in alloxan induced diabetic mice was only about 19% of that seen in normal mice. Further glucokinase enzyme activity was decreased by more than 90% because some immunoreactive glucokinase enzyme may have abnormal function. Moreover hepatic glycogen

Table 1: Curative effect of crude and methanolic extract of *C. longa* on Glucose, ALT, AST and AOA in CDG, CTG and MTG at different time intervals

	CDG	CTG	MTG	CDG	CTG	MTG
	Glucose (mg/dl)			AOA (mmol/l)		
Normal (a)	109.17±3.44	107.5±3.46	116.83±2.21	2.1±0.124	2.07±0.178	2.13±0.161
8 days after Alloxan inj (b)	242.17±6.19*** ↑ (121.83%)	218.83±6.25*** ↑(103.6%)	290.17±23.49*** ↑(148.37%)	0.713±0.053*** ↓ (66.05%)	0.91±0.041*** ↓(56.04%)	0.70±0.08*** ↓(67.14%)
4 hrs (c)	267±6.22*** ↑ (144.57%)	163.67±11.72*** ↓(25.21%)	183.83±18.48*** ↓(36.65%)	0.69±0.061*** ↓ (67.14%)	1.9±0.12*** ↑(108.79%)	1.12±0.05*** ↑(60%)
8 hrs (d)	289.5±6.65*** ↑ (165.18%)	138.83±3.98*** ↓(36.56%)	155±4.48*** ↓(46.58%)	0.71±0.068*** ↓ (66.19%)	1.3±0.08*** ↑(42.86%)	1.35±0.06*** ↑(92.86%)
24 hrs (e)	243.3±6.79*** ↑ (122.86%)	129.83±3.79 *** ↓(53.93%)	134.17±3.75*** ↓(53.76%)	0.68±0.074*** ↓ (67.62 %)	1.03±0.16*** ↑(13.19%)	2.84±1.64*** ↑(92.86%)
	AST (U/l)			ALT (U/l)		
Normal (a)	10.17±0.87	10.33±1.054	8.9±0.795	10.33±1.48	11.08±10.28	9.03±0.877
8 days after Alloxan inj (b)	65.5±7.62*** ↑(544.05%)	71.17±7.04*** ↑(588.96%)	69.33±6.09*** ↑(678.99%)	69.67±7.82*** ↑(574.44%)	65.67±8.24*** ↑(492.69%)	70±7.87 *** ↑(675.19%)
4 hrs (c)	65.3±7.44*** ↑(542.09(%)	33.82±3.28*** ↓(52.48%)	35.33±3.17*** ↓(49.04%)	70.17±6.28*** ↑(579.28%)	39.05±5.94 ** ↓(40.53%)	31.5±4.14 *** ↓(55%)
8 hrs (d)	64.17±7.40*** ↑ (530.97%)	25.27±2.65*** ↓(64.49%)	21.83±1.64*** ↓(68.51%)	67.83±8.09*** ↑(556.63%)	25.13±1.68*** ↓(61.73%)	25.5±2.43*** ↓(63.57%)
24 hrs (e)	63.33±8.37*** ↑ (522.71%)	21.67±2.22*** ↓(69.55%)	17±2.07*** ↓ (75.47%)	68.5±7.65*** ↑(563.12%)	22.45±2.09*** ↓(65.81%)	18.95±0.72*** ↓(72.93%)

CDG: This group was injected alloxan monohydrate**,** 180mg/kg b.w i.p and then kept orally on 2% acacia solution
CTG: This group was given crude drug suspension, 2g/kg b.w given orally, 8 days after alloxan injection and glucose, AOA, ALT and AST were observed at 4, 8 and 24 hrs after drug administration.
MTG: This group was given crude drug methanolic extract suspension equivalent to 2g/kg b.w of crude drug orally 8 days after alloxan injection and glucose, AOA, ALT and AST were observed at 4, 8 and 24 hrs after drug administration.
Values are expressed as mean ± S.E
For CDG **b, c, d** and **e** are compared with a for t-test
For CTG and MTG, **a** is compared with **b** and **c, d** and **e** are compared with **b** for t test
***Highly significant P<0.005, **Very significant P< 0.01, *Significant P<0.05, ^{NS}Non-significant P>0.05
% change is in parenthesis

is also decreased in alloxan induced diabetic mice. Because of the metabolic disorder caused by diabetes, changes in the serum transaminases may occur, indicating tissue damage by toxicants. So it could be suggested that *C. longa* and its extract improves and protects glucokinase and inhibits reduction in hepatic glycogen in liver therefore it reduces serum transaminases (Zhang *et al.,* 2007; Sakurai and Ogiso, 1995; Takasun *et al.,* 1991; Munday, 1988; Heikikila *et al.,* 1976).

In the present investigation the studies have been planned to see the curative and the protective action of the *C. longa* and its methanolic extract in alloxanized and normal rabbits. Tetrahydrocurcumin a major metabolite of curcumin has also been assessed for its effect on hepatic and renal markers and proteins in type 2 diabetic rats (Murugan and Pari, 2007). It was observed that tetrahydrocurcumin also protects against renal and hepatic damage in diabetic condition. In the present study serum glucose, ALT and AST were assessed and it was observed that *C. longa* given in a dose of 2g/kg when given to diabetic rabbits improved these parameters as compared to the untreated diabetic rabbits. In curative group the crude and methanolic extract of *C. longa* reduced glucose ALT and AST and significant results were obtained. In both protective groups rabbits pretreated with crude and methanolic extract of *C. longa* highly significant results were obtained in the parameters observed, so it could be suggested that metabolite tetrahydrocurcumin provides long term protective effect in liver and pancreas. In diabetes insulin deficiency results in to reduce synthesis of proteins and cause glycation which results into the formation of oxygen derived free radicals. *C. longa* and its methanolic extracts reduce the blood glucose level by reducing the influx of glucose through polyol pathway (Arun and Nalini, 2000).

Therefore it can be suggested that *C. longa* and its methanolic extracts significantly reduced glucose by increasing glucose utilization in erythrocytes or by inhibiting or blocking the enzymes that convert the dietary carbohydrates into sugar. It improves and protects the level of glucokinase both in liver and pancreas; therefore it reduces serum transaminases by maintaining the primary enzyme glucokinase level found both in liver and pancreas. *C. longa* reduces glucose level and serum

Table 2: Protective effect of crude and methanolic extract of *C. longa* on Glucose, AOA, ALT and AST in normal and alloxan treated rabbits.

	PCG	PCD	PMG	PCG	PCD	PMG
	Glucose (mg/dl)			AOA (mmol/l)		
1st day (a)	115.33±3.56	116.33±2.38	119.5±2.75	2.25±0.19	2.38±0.06	1.4±0.08
8th day (b)	114±5.24 NS	115.5±1.61 NS	118.33±1.73 NS	2.25 ±0.18 NS	2.6±0.07**	1.75±0.043***
9th day (c)	106.5±6.35 NS ↓(7.65%)	111.3±7.72 NS ↓(4.32%)	110.67±3.90* ↓(7.39%)	1.53±0.15** ↓(32%)	1.88±0.03*** ↓(21%)	1.58±0.04*↓ (12.9%)
17th day (d)	213±7.86*** ↑(84.68%)	166.7±11.05*** ↑(43.3%)	137±3.79*** ↑(14.6%)	0.91±0.05*** ↓(59.56%)	1.46±0.03*** ↓(38.66%)	1.42 ±0.03 NS
24th day (e)	257.4±14.21*** ↑(123.19%)	181.33±16.12*** ↑(55.85%)	179.67±7.14*** ↑(50.35%)	0.44±0.015*** ↓(80.35%)	1.33±0.05*** ↓(44.12%)	1.28 ±0.05 NS
	ALT (U/l)			AST (U/l)		
1st day (a)	14.5±1.38	16.67±1.43	17±1.15	11.33±0.88	14.67±1.33	15.83±1.25
8th day (b)	15.5±0.96 NS	12.17*±1.58	14.5±1.31 NS	11.33±0.72 NS	13.17±1.01 NS	14.3±0.56 NS
9th day (c)	33.88±2.45*** ↑(133.7%)	31.67±2.79*** ↑(89.98%)	21.83±0.75*** ↑(28.41%)	32.7±2.45*** ↑(188.61%)	27±1.97*** ↑(84.05%)	20.33±0.88** ↑(28.43%)
17th day (d)	65.42±7.32*** ↑(351.17%)	33.83±4.38*** ↑(102.9%)	30.7 ±1.385*** ↑(85.29%)	56.17±8.49*** ↑(395.76%)	32.67±8.49*** ↑(122.7%)	30.67±1.12** * ↑(93.75%)
24th day (e)	69±6.89*** ↑(375.9%)	36.5±3.94*** ↑(118.96%)	35±1.84*** ↑(105.9%)	61.2±6.88*** ↑(440.16%)	33.5±1.45*** ↑(128.36%)	41±1.39*** ↑(159%)

PCG: PCG was kept on 2% acacia solution initially for 8 days and injected alloxan intraperitoneally (180 mg/kg b.w) along with PCD and PMG and the changes in glucose, ALT and AST were observed on 9th, 17th and 24th day.
PCD: The PCD was given crude drug suspension (dose: 2g/kg b.w) prepared freshly, once daily for 8 days. On the 8th day alloxan (180 mg/kg b.w) was injected intraperitoneally and the changes in glucose, ALT and AST were observed on 9th, 17th and 24th day.
PMG: The PMG was given suspension of methanolic extract of crude drug (dose: equivalent to 2g/kg b.w of crude drug) prepared freshly, once daily for 8 days. On the 8th day alloxan (180 mg/kg b.w) was injected intraperitoneally and the changes in glucose, ALT and AST were observed on 9th, 17th and 24th day.
Values are expressed as mean ± S.E
For PCG **b, c, d**, and e are compared with **a** for t test
For PCD and PMG b is compared with **a**, and **c, d** and **e** are compared with **a** for t test.
***Highly significant P< 0.005, **Very significant p< 0.01, *Significant P< 0.05, NSNon-significant P> 0.05
(% change is in parenthesis)

transaminases to almost 50% with a dose of 2g/kg b.w. It was also observed that overall methanolic extract produced better effect as compared to the crude *C. longa*. *C. longa* contains 3 to 4% of curcumin which can neutralize free radicals formed in the body. With the prevention of formation of free radicals *C. longa* prevents the damage caused by alloxan to pancreas. Turmerin, water soluble protein found in *C. longa* exhibits antioxidant capacity and it was observed that this protein also inhibits α-amylase and α-glucosidase activities (Lekshmi *et al.,* 2011). In another study it was observed that the curcumin diet lowered liver weight and lipid peroxidation in plasma and urine. *C. longa* and its extract may also scavenge superoxide radical production and inhibit glycosylation of proteins which contribute to the pathogenesis of cellular dysfunction and hence improve the metabolic state of diabetic patient. Curcumin, yellow component in *C. longa* reduces plasma free fatty acid, cholesterol, and triglyceride concentrations and increased the hepatic glycogen and skeletal muscle lipoprotein lipase in db/db mice. Curcumin also normalized erythrocyte and hepatic antioxidant enzyme activities (superoxide dismutase, catalase, gluthathione peroxidase) in db/db mice that resulted in a significant reduction in lipid peroxidation (Seo *et al.,* 2008). Hence it can be concluded that *C. longa* and its extract improves the metabolic status of diabetic patient because of its antioxidant and free radical scavenging properties.

C. longa needs more attention from the researchers to further evaluate its safety in different diseased conditions to make its use more common in humans. Natural drugs are more safe with little side effects hence they should be made available to common people in proper dosage form to heal the humanity.

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