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ANTIDIABETIC PROPERTIES OF METHANOLIC EXTRACTS OF *CARISSA CARANDAS* L. FRUITS IN ALLOXAN-INDUCED DIABETIC MICE

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ABSTRACT

In many Asian countries such as India, Bangladesh, Myanmar and Malaysia, *Carissa carandas* L., is commonly used as healing plant to treat diabetes. The purpose of the presents study was to assess the therapy effect of *Carissa carandas* L., fruit extract on glucose level, total cholesterol, triglyceride, LDL-cholesterol, and HDL-cholesterol in blood serum of diabetic animals. Hyperglycemia was induced by an injection of alloxan monohydrate 150 mg/kg (i.p.). After 72 hr, the mice having Blood Glucose Level (BGL) greater than 13.9. mmol/dL were selected for the investigation. BGL was monitored after 1, 5, 10, 15 and 21 days and compared with Glibenclamide (5 mg/kg). A Methyl alcohol extract of *Carissa carandas* L., fruit was daily administered orally by gavages to mice in doses of 50 and 100 $mgkg^{-1}$ b.w. for 21 days to evaluate the antidiabetic and antihyperlipidemic effects on normal and alloxan-induced diabetic mice. Levels of blood serum glucose, total cholesterol, triglyceride and LDL-cholesterol decreased in alloxan-induced diabetic mice after treatment with 50 and 100 $mgkg^{-1}$ b.w. methyl alcohol fruit extract of *Carissa carandas* L., for 21 days. The study validates scientifically the widely claimed use of *Carissa carandas* L., fruit as an ethnomedicine to treat diabetes mellitus.

KEYWORDS: Antidiabetic; *Carissa carandas*; Diabetes mellitus; Hyperglycemic; Hyperlipidemic.

INTRODUCTION

Diabetes is a chronic disease resulted either from insufficient insulin production by pancreas or the body's ineffective use of insulin. There are more than 220 million diabetic people worldwide, and it was estimated in 2005 that 1.1 million people died from diabetes with almost 80% of these deaths occurred in low- and middle-income countries.[1] WHO projected that death caused by diabetes will double between 2005 and 2030.

Realizing the seriousness of this scenario, researchers worldwide are putting endless efforts in searching for complementary and alternative medicine therapies (CAM) for diabetes. Herbs, dietary supplements, and mind-body medicine are the most commonly used and studied CAM modalities to treat diabetes.[2] *Allium sativum* (garlic), aloe vera, *Coccinia cordifolia* (ivy gourd), *Gymnema sylvestre* (gymnema), *Momordica charantia* (bitter melon), *Opuntia streptacantha* (prickly pear cactus), *Panex ginseng* (ginseng) and *Trigonella foenum graecum* (fenugreek) are some examples of herbs and biologically based practices used for diabetes.[2] *Vitex Nigundo* leaf,[3] *Tamarindus indica*,[4] *Vigna unguiculata*,[5] *Artemisia herba alba*,[6] *Aegle marmellos* leaf,[7] *Moringa oleifera*,[8] *Morus alba* fruit,[9] and *Ortosiphon stamineus* leaf,[10] were proven scientifically in diminution of fasting blood sugar level of diabetic animals.

Carissa carandas Linn. belonging to family *Apocyanaceae* is a large aeromatic shrub distributed throughout India, Bangladesh, Malaysia, Indonesia and Thailand, as well as in tropical areas. *Carissa carandas* is large dichotomously branched evergreen shrub with short stem and strong thorn in pairs. This species is a rank-growing, straggly, woody, climbing shrub, usually growing to 10 or 15 ft (3–5 m) high, sometimes ascending to the tops of tall trees. Traditionally, whole plant and its parts were used in the treatment of various ailments. The roots were employed as a bitter stomachic, vermifuge and as an ingredient in the remedy for itches. The roots were reported to contain salicylic acid and cardiac glycosides. It also contains carissone; d-glycoside of-sitosterol; glucosides of odoroside H; carindone; a terpenoid lupeol; ursolic acid and its methylester; also, carinol, a phenolic lignan. Fruits

contains good amount of vitamin C. The fruits, leaves and bark are rich in tannins,[11] In Ayurveda, the unripe fruits were used as an anthelmintic, astringent, appetizer, antipyretic, antidiabetic, aphrodisiac, in biliary disorders, stomach disorders, rheumatism and diseases of the brains.[11,12] It is useful in treat-ment of diarrhea, anorexia and intermittent fevers. Fruits have also been studied for its analgesic, anti-inflammatory and lipase 1 activities.[13] It is used by tribal healers of Western Ghat region of Karnataka, India as hepatoprotective and antihy-perglycemic. However, no scientific data is available to validate the folklore claim.[14,15] Keeping the above information in view, this research was aimed to study the efficacy of fruit extract of *Carissa carandus* in antihyperglycemic and antihyperlipidemic responses in severe diabetic mice.

MATERIALS AND METHODS

Plant material

Unripe fruits of *Carissa carandas* L. were collected from Kazla, Rajshahi, Bangladesh, in June, 2018. Plant specimen was authenticated by Professor Dr. A.H.M. Mahbubur Rahman, Department of Botany, University of Rajshahi, Bangladesh. A voucher specimen number # 132 was deposited to the herbarium in the Department of Botany, University of Rajshahi. The fruits were cut into small pieces, sun dried and ground into powder with an electrical grinder. The powder was packed and stored in a refrigerator at 4 °C until used.

Extraction of fruit

Extraction was performed by mixing 60 g powder with 600 ml of 95% ethyl alcohol with occasional shaking and stirring in a shaker for 5 days. The resulting extract was filtered by using a muslin cloth and ethyl alcohol was allowed to evaporate for 5-7 days. The crude extracts of the plants were then lyophilized to powder and stored at 4 °C for further experiments.

Experimental animal

Swiss albino mice of same age with body weight of about 20-25 gm were collected from the International Cholera and Dysentery Disease Research, in Dhaka, Bangladesh (ICDDRB) for the experiments. The mice were housed in cages (six mices per cage) at ambient temperature of 25-30 °C and 45-55% relative humidity with a 12 h each of dark and light cycle for one week prior to the experimental work. The mice had free access to standard pellet diet and water *ad libitum*. The institutional Animal Ethics Committee had approved this study.

Induction of diabetes mellitus

Diabetes was induced in overnight fasted mice by a single intraperitoneal injection of Alloxan (150 mg/kg body weight) in a 0.1 M sodium citrate buffer (pH 4.5). The age-matched control mice received an equivalent amount of citrate buffer. Food and water intake were closely monitored daily after Alloxan administration. The development of hyperglycemia in mice was confirmed by fasting (16 hour) blood glucose measurement in the tail vein blood, 72 hours after Alloxan administration, with a portable glucometer (Accu-Chek, Roche, Germany). The animals with fasting blood glucose level 13.9 mmol/L with other symptoms of diabetes mellitus such as polyphagia, polydipsia, polyuria, and weight loss were considered as diabetic and included in the study.

Experimental design

A long-term study of 21 days was conducted in the severely diabetic mice. Twenty mice were divided into five groups of four mice each. The mice grouping was as follow

Group-I (Normal control): Mice feed with standard pellet diet and water

Group-II (Diabetic control): Diabetic mice without treatment.

Group-III (Treated-2): The diabetic mice treated with methanol extract of *Carissa carandas* fruits (MECC) at a dose of 100 mg/kg body weight for 21 days.

Group-IV (Treated-1): The diabetic mice treated with methanol extract of *Carissa carandas* fruits (MECC) at a dose of 50 mg/kg body weight for 21 days.

Group-V (Positive control): Diabetic mice were treated by Glibenclamide at dose of 5 mg/kg body weight.

Before giving the supplement of *Carissa carandus* extract, the basal blood glucose levels were measured in all the groups. After 21 days of experiment, all mice were anaesthetized under chloroform vapor and blood samples were withdrawn by cardiac puncture. Aliquots of blood were poured into screw-capped bottles containing fluoride/oxalate anticoagulant for determination of blood serum glucose, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, SGPT and SGOT. All analyses were carried out within 24 hour of blood collection.

Analytical procedure

Blood glucose concentration was estimated according to the glucose oxidase method using a reagent kit (Randox Laboratory Ltd., UK). Serum total cholesterol and HDL-cholesterol concentrations were measured according to CHOD-PAP method using a commercial kit. Serum LDL-cholesterol concentration was also estimated by CHOD-PAP method after precipitation with magnesium sulphate and phosphotunstic acid. Levels of ALT and ASP activity were estimated by using SGPT and SGOT assay kit, respectively. Triglyceride concentration was measured by GPO-PAP method using a commercial kit.

Statistical analysis

The statistical differences represented by letters were obtained through one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD), post hoc test ($P<0.05$). Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations ($P<0.001$). These were carried out using Microsoft office Excel 2010 and SPSS

version 16.0 program (IBM Corporation, New York, USA). The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD).

RESULTS

Effect on body weight of non-diabetic and diabetic mice

Table 1 describes the changes of bodyweight of normoglycemic and alloxan-induced diabetic mice. There was significant body weight decreased in alloxan-induced diabetic mice as compared to that of non-diabetic mice throughout the 21 days study period.

Body weight of the diabetic mice increased after administration of *Carissa carandus* fruit extract for 21 days. It was observed that the body weight of the diabetic mice treated with the fruit extract at a dose of 50 $mgkg^{-1}$ b.w. increased by 4.7% from 120.5 g to 126.13 g. An increase of 5.9% in body weight was observed in the diabetic mice treated with the fruit extract at a dose of 100 $mgkg^{-1}$ b.w., while the body weight of the diabetic mice without treatment decreased by 0.8% from 150.2 g to 120.1 g.

Effect on blood glucose level and serum enzyme activity

The plant extract produced significant changes in the blood glucose level in alloxan induced diabetic mice (Figure 1). Plant extract administered mice showed significant (P<0.001) reduction of blood glucose level in both concentration of 50 mg/kg and 100 mg/kg body weight in diabetic mice group. This level of reduction was as near as glibenclamide administered mice. In 5^{th} to 21^{st} days, MECC at both doses (50 mg/kg and 100 mg/kg body weight) lowered the glucose level by 11.93% - 46.66% and 17.34% - 54.67%, respectively than the diabetic control group.

Effect on cholesterols and triglyceride levels

Figure 2 showed the serum levels of Total cholesterol (TC), Triglycerides (TG), LDL, VLDL, HDL and hyper cholesterol of control and alloxan-induced diabetic mice. All lipid except HDL parameters showed in significantly different at P<0.001. Reduction of Total Cholesterol (TC) level was 12.06% (in 50 mg/kg bw.) and 23.55% (in 100 mg/kg bw.) observed in *C. carandas* fruits (MECC) extract treated diabetic mice group whereas in positive control group reduction was 35.66%. In 21 days, observation the treatment groups showed significant decrease (P<0.001) of total cholesterol compared with the diabetic control group. The Levels of triglyceride in the diabetic group were increased after 21 days of alloxan-induction.

Serum triglyceride of treatment group of mice was lower than that of diabetic control. In Figure-2 the diabetic control group showed an increase in LDL levels higher than the (normal) control group. LDL level was significantly reduced (P<0.001) with *C. carandas* treatment at 19.36% (in 50 mg/kg BW) and 43.75% (in 100 mg/kg bw.). VLDL level was significantly reduced (P<0.001) with *C. carandas* treatment at 10.09% (in 50 mg/kg bw.) 21.23% (in 100 mg/kg bw.), whereas the HDL level increased by 11.79% (in 50 mg/kg bw.) and 21.67% (in 100 mg/kg bw.). Glibenclamide (5 mg/kg b w.) treated mice showed a reduction of TG by 30.40%, LDL by 78.53%, VLDL by 30.39% and an increase in HDL by 31.77%.

Effect on serum enzyme activity

There was a significant (P<0.001) increase of SGPT and SGOT level (figure after diabetes induction which was compensated by *C. carandas* fruits (MECC) significantly (P<0.001). The percent of lowering of SGPT by *C. carandas* fruits (MECC) from diabetic control groups were 26.08%-31.92%, whereas 38.48 % for glibenclamide. The reduction of SGOT level was highly significant (P<0.001) for *C. carandas* fruits (MECC) at 17.23%-27.74%, whereas 33.55% for glibenclamide.

Table 1: Effects of methyl alcohol fruit extract of *Carissa carandus* L. on the body weight in Alloxan induced diabetic mice.

Groups (treatment and doses)	Body weight (g)				
	0 day	5 day	10 day	15 day	21 day
I (Normal control)	135.4 ± 8.1	141.5 ± 3.1	150.5 ± 4.2	155.1 ± 5.0	160.3 ± 5.1
II (Diabetic control)	150.2 ± 3.2	143.4 ± 9.1	135.6 ± 5.1	130.1 ± 1.2	120.1 ± 3.1
III (extract, 50 $mgkg^{-1}$ b.w.)	120.5 ± 6.1**	121.2 ± 5.2**	123.3 ± 5.4**	124.3 ± 2.7**	126.1 ± 3.7**
IV (extract, 100 $mgkg^{-1}$ b.w.)	155.4 ± 4.3**	158.9 ± 4.4**	162.8 ± 4.8**	165.2 ± 1.2**	168.1 ± 3.2**
V (Glibenclamide, 0.5 $mgkg^{-1}$ b.w.)	153.1 ± 8.7	154.1 ± 5.1	155.3 ± 6.1	156.3 ± 9.2	158.3 ± 2.2

Values were expressed as mean ± SD. Body weight in the treated mice were significantly different from diabetic control group at **P<0.001 and *P<0.05. MECC indicates methanol extract of *C. carandas* unripe fruits.

Table 2: Effects of methyl alcohol fruit extract of *Carissa carandus* L. (MECC) on serum glucose level in Alloxan induced diabetic mice.

Groups (treatment and doses)	Serum glucose(mmol/dl)				
	0 day	5 day	10 day	15 day	21 day
I (Normal control)	5.97± 0.14	5.32 ± 0.24	5.72 ± 0.20	5.39 ± 1.05	5.44±0.41
II (Diabetic control)	20.83±0.81	20.92±0.85	22.54±0.10	23.64±1.17	25±0.61
III (MECC, 50 $mgkg^{-1}$ b.w.)	20.5±0.52*	18.43±0.30**	16.38±0.11**	14.9±0.20**	13.33±0.15**
IV (MECC, 100 $mgkg^{-1}$ b.w.)	19.8±0.7*	17.3±0.36**	14.93±0.55**	13.16±0.90**	11.33±0.55**
V (Glibenclamide, 0.5 $mgkg^{-1}$ b.w.)	21.09±0.40	16.32±0.08	13.45±0.52	10.64±0.14	9.40±0.2

Values were expressed as mean ± SD. Blood glucose level in the treated mice were significantly different from diabetic control group at **P<0.001 and *P<0.05. MECC indicates methanol extract of *C. carandas* unripe fruits.

Table 3: Effects of MEMA fruits on biochemical parameter in diabetic mice.

Group (treatment and doses)			Parameters		
	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I (Normal control)	115.50±0.81	115.73±0.15	85.23±0.25	7.45±0.71	23.14±0.031
II (Diabetic control)	190.40±0.98	179.87±0.20	137.54±0.22	16.88±0.84	35.97±0.042
III (MECC, 50 $mgkg^{-1}$ b.w.)	167.4±0.05**	162.23±0.25**	121.33±0.35**	13.62±0.20**	32.45±0.05**
IV (MECC, 100 $mgkg^{-1}$ b.w.)	145.57±0.35**	141.67±0.32**	107.73±0.21**	9.50±0.57**	28.33±0.06**
V (ibenclamide, 0.5 $mgkg^{-1}$ b.w.)	122.50±0.45	125.18±0.43	93.84±0.15	3.62±0.39	25.04±0.09

Values were expressed as mean ± SD. Biochemical parameters of lipid profile in the treated mice were significantly different from diabetic control group at **P<0.001. MECC indicates methanol extract of *C. carandas* fruits.

Table 4: Effect of MECC fruits on serum SGPT and SGOT of experimental mice.

Group (treatment and doses)	SGPT(U/L)	SGOT(U/L)
I (Normal control)	67.80±0.61	79.88±0.60
II (Diabetic control)	119.91±0.67	135±0.66
III (MECC, 50 $mgkg^{-1}$ b.w.)	88.63±0.76**	111.74±0.95**
IV (MECC, 100 $mgkg^{-1}$ b.w.)	81.64±0.59**	97.55±0.87**
V (Glibenclamide, 0.5 $mgkg^{-1}$ b.w.)	73.77±0.48	89.7±0.84

SGPT & SGOT level in the treated mice were significantly different from diabetic control group at **P<0.001. MECC indicates methanol extract of *C. carandas* fruits.

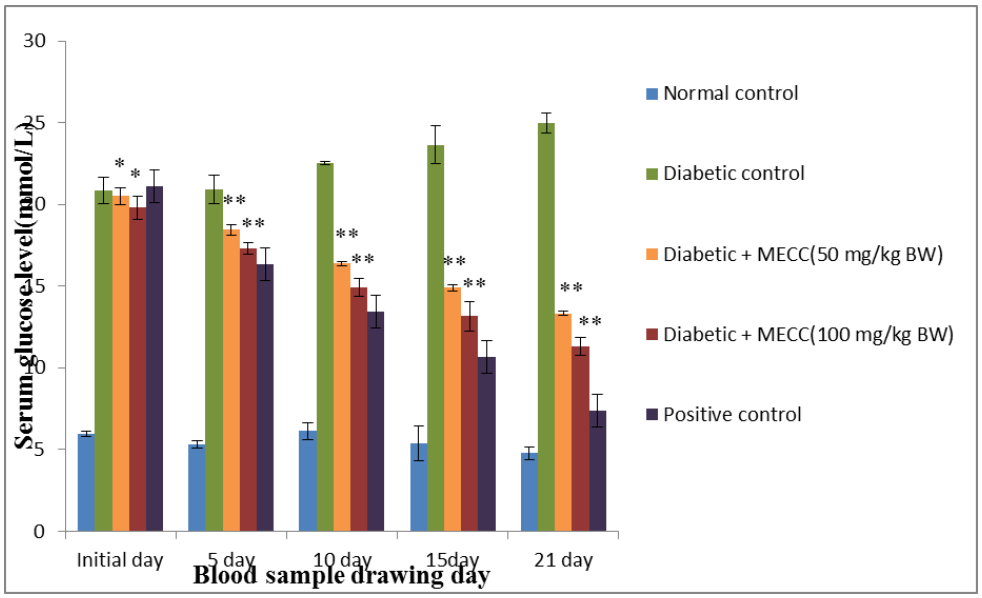

Figure 1: Change of blood glucose level after Methanol extract of *C. carandas* fruits (MECC) treatment in diabetic mice.

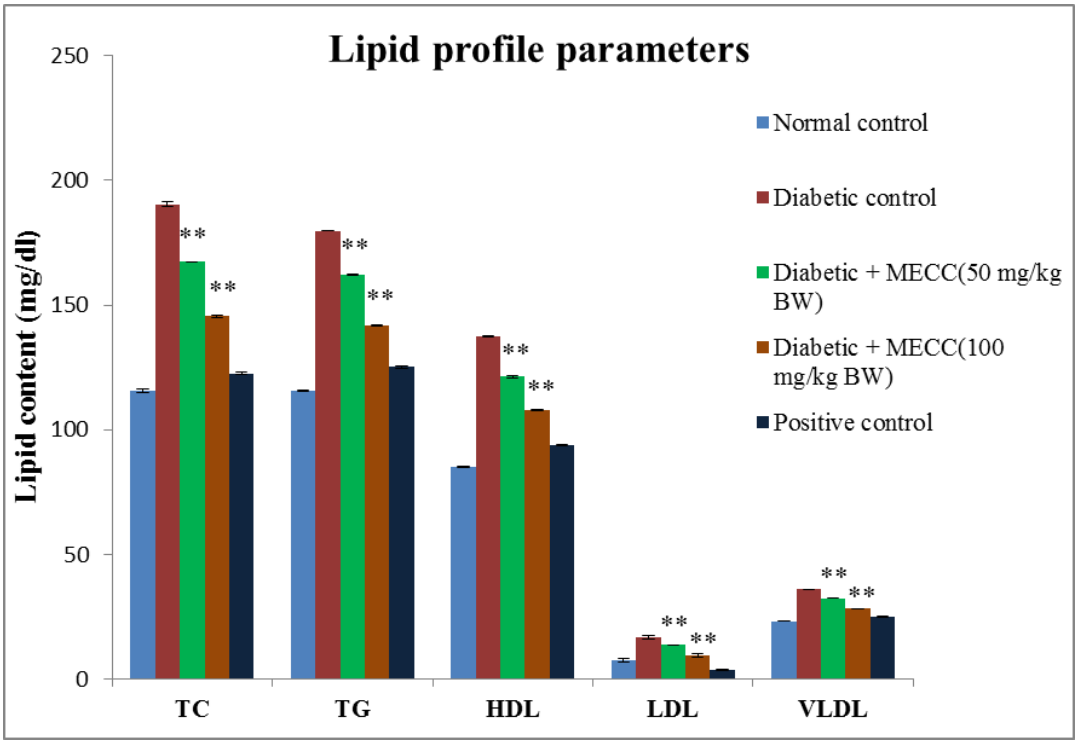

Figure 2: Effects of Methanol extract of *C. carandas* fruits (MECC) on lipid profile of diabetic mice after 21 days treatment.

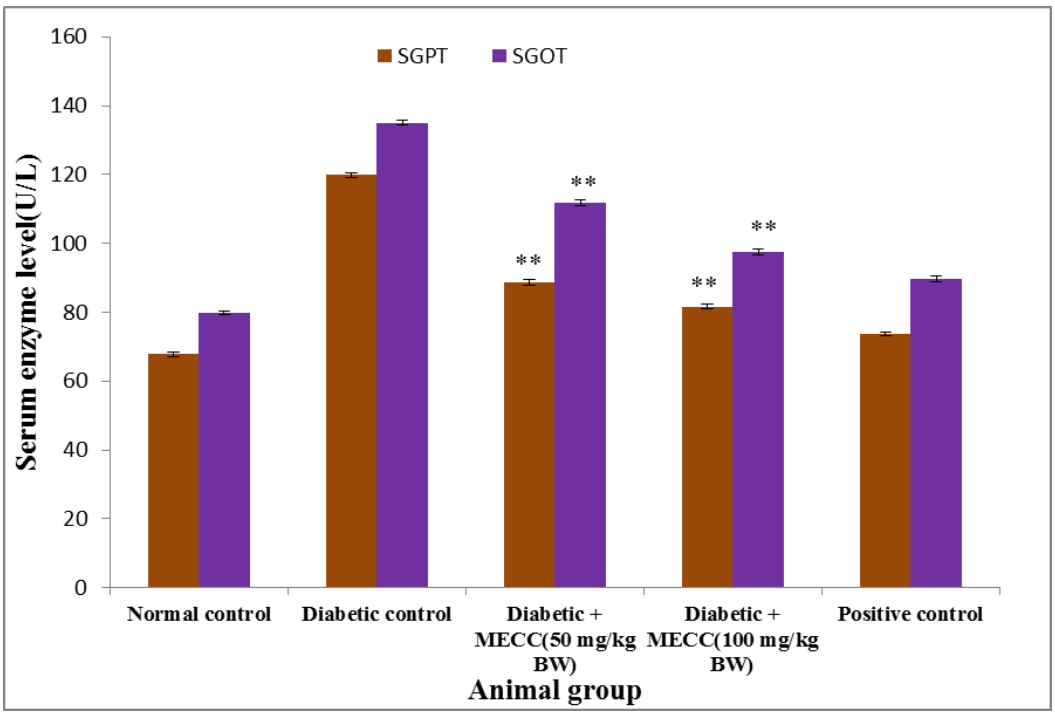

Figure 3: Effect of *C. carandas* fruits (MECC) on serum SGPT and SGOT in diabetic mice after 21 days treatment.

DISCUSSION

In this study, diabetes was induced in mice by the injection of Alloxan (80 mg/kg bw.) intraperitoneally. Alloxan is a beta cytotoxin agent which destroys β-cell of islet of Langerhans of pancreas of animal resulting in the reduction of release of insulin which leads to increase in blood glucose level.[16] In diabetes mellitus, Hypercholesterolemia and hypertriglyceridemia are common complications in addition to hyperglycemia.[17] This work was done to evaluate the effects and comparison of methanolic extract of C. carandas Linn. fruits extract at the dose of 50 mg/kg b.w. and 100 mg/kg b.w. on body weight, blood glucose, serum total cholesterol (TC), serum triglyceride (TG), serum HD L,

LDL and serum enzymes (SGPT, SGOT) in Glibenclamide-induced diabetic mice

Significant decrease in body weight of diabetic mice was observed in this study. However, this is a normal observation as Alloxan-induced diabetes is characterized by a severe loss in body weight.[9] This is possible due to catabolism of proteins in the absence of insulin.[18] Alloxan is a beta cytotoxin agent which destroys β-cell of islet of Langerhans of pancreas of animal resulting in the reduction of release of insulin which leads to increase in blood glucose level.[16] There were significant decreases in blood glucose level in alloxan induced diabetic mice treated with C. carandas L. fruit extract for entire period of the experiment. The alcoholic extract of fruit was administered at a dose of 50 mg/kg bw. and 100 mg/kg bw. After 21 days of treatment with alcoholic extract at dose of 100 mg/ kg, the Blood Glucose Level (BGL) decreases from 19.80 to 11.10 mg/dL. The results in decrease in BGL comparable with standard drug glibenclamide which decrease BGL from 21.09 to 10.40 mg/dL. However, the blood glucose levels of diabetic mice treated with the methanol extract were similar with the standard treated group mice. This is due to the stronger extraction capacity of methanol which could have produced a greater number of active components responsible for blood glucose-lowering activity. In the present study, the methanolic extracts from C. carandas L. fruit showed a significant hypoglycemic effect in the alloxan-induced diabetic mice. Therefore, the mechanism of the extract is possibly an insulin-independent mechanism. Alloxan induces diabetes by pancreatic cell damage mediated through the generation of oxygen-free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation.[19]

Results of the present studies confirmed that, insulin deficiency as well as an increase in blood glucose level increase cholesterol and triglyceride levels due to the fat storage into liver. Treatment with methanolic extract may improve insulin level by unknown mechanism that will reduce the storage of fat in the liver. The liver, that is a major organ is affected by diabetes. If liver enzyme activity increased, it may be due to the result of liver damage.[17] Liver enzymes are good indicators for the liver functioning. Diabetic conditions also caused secretion & release of liver enzymes to the blood circulation due to destruction of cells by the change in membrane structure.[20] Oral administration of methanolic extracts in 50 and 100 mg/kg bw. doses significantly reduce the enzyme level in blood.

CONCLUSION

The present study revealed that the methyl alcohol extract of *Carissa carandus* fruit has significant antihyperglycemic and antihyperlipidemic potential in treating severe diabetic mice. A more pronounce effect is anticipated if a longer treatment is administered to the diabetic mice. Further biochemical investigation is underway to ascertain the active compound in the extract.

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