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Research Article

COMBINED EFFECT OF *COCCINIA INDICA* (WIGHT & ARN) AND *SALVADORA OLEOIDES* (DECNE) ON BLOOD GLUCOSE LEVEL AND OTHER RISK FACTORS ASSOCIATED WITH TYPE 2 DIABETES MELLITUS IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus (DM) is a heterogeneous metabolic disorder characterized by common feature of chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism and often associated with various complications. Combinations of two or more plants in the treatment of severe diseases have been beneficial. The present study reports the combined effect of *Coccinia indica* (Wight & Arn) and *Salvadora oleoides* (Decne) leaf extract on blood glucose level and certain other biochemical parameters in alloxan induced diabetic rats. The Combined Methanolic Extract (CMEt) of the two plants at a dose level of 150 mg/Kg showed significant ($p<0.01$) reduction in blood glucose level of diabetic rats compared to that of standard drug Glipizide (5 mg/kg body weight). CMEt also showed significant ($p<0.01$) effect on lipid profile, ALS/AST activity, and serum creatinine and urea levels, there by exhibiting its overall significant antidiabetic potential.

Keywords*: Diabetes mellitus*, Antidiabetic, *Coccinia indica, Salvadora oleoides*, Alloxan, Glipizide.

INTRODUCTION

Diabetes is prevalent worldwide and is stated to be one of the important causes of death worldwide. Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion insulin action or both[1]. The countries with the largest number of diabetic people in the year 2025 will be India, China and United States[2]. The risk of diabetic complications, particularly cardiovascular diseases (CVD) peripheral vascular disease (PVD)[3]. Complications such as coronaryartery disease (CAD), stroke, neuropathy, renalfailure, retinopathy amputations, and blindness etcare known to be associated with DM[4].Herbal medicines are the oldest remedies known to mankind which have been used by all cultures but India having one of the oldest, richest and diverse traditions associated with the use of medicinal plants[5].The role of herbal plants in the treatment of diabetes is already known and much of them have been scientifically established.

World Health Organization expert committee on diabetes has listed as one of its recommendations that traditional methods of treatment for diabetes should be further investigated[6].Many herbal medicines as single agents or in different oral formulations have been recommended for diabetes mellitus due to the fact that they are less toxic than oral hypoglycemic agents such as sulfonylureas, metformin etc[7].

Coccinia indica Wight and Arnold (Cucurbitaceae)commonly known as 'Ivy gourd' and 'Kundru' in Hindi is aperennial tendril climber, available in wild and cultivated form. It is the native of Central Africa, India and Asia and distributed naturally in China, Tropical Asia, India, Australia and Africa. It is considered as a valuable wild vegetable by the indigenous people of Southeast Asia and India. Hypoglycemic[8] and antidiabetic[9]potential of *Coccinia indica* has been established.

*Salvadora oleoides (*Decne) (Salvadoraceae family), commonly known in India as *meethajal*is an oil yielding medicinal and multipurpose tree. It can grow in arid and alkaline conditions. The leaves of *S. oleoides* are used to relieve cough and for treatment of enlarged spleen and fever. The leaves of *S. oleoides* are said to possess anti-inflammatory, analgesic, and antiulcer activity.[10]Recently anti-hyperlipidemic potential of the methanolic extract of its leaves have been reported.[11]In the present study we have reported the combined effect of methanolic extracts of *Coccinia indica* and *Salvadora oleoides*on blood glucose level and certain other biochemical parameters in Alloxan induced diabetic rats.

MATERIAL AND METHODS

Reagents and Chemicals

Alloxan monohydrate was purchased from Loba Chemie, India. Blood glucose estimation and other biochemical parameters were estimated using Siemens Diagnostic. Other solvents and chemicals were purchased from Rankem India Pvt Ltd and were of AR grade.

Plant material

Leaves of *Coccinia indica* (Wight & Arn) were collected from locality of Patna, India and were authenticated by National Institute of Science Communication and Information Resources (NISCAIR-CSIR), New Delhi, India with reference number NISCAIR/RHMD/Consult/2010-11/1676/274. Leaves of *Salvadora oleoides* (Decne) were collected from New Delhi, India and were authenticated by National Institute of Science Communication and Information Resources (NISCAIR-CSIR), New Delhi, India with reference number NISCAIR/RHMD/Consult/2010-11/1675/273.

Leaves of *Coccinia indica* & *Salavadora oleoides*were dried under shade and powdered. 500 gms of powdered material were subjected to soxhlet extraction with petroleum ether (60 – 80°C) followed by extraction with methanol. The extracts were then evaporated and dried.

Animals

Adult albino rats of both sexes weighing 250-350 gms were procured from disease free CPCSEA approved Institutional animal house (Reg. no. 273/CPCSEA) of SBSPGI, Dehradun. They were kept at Institutional animal house in standard polypropylene cages and maintained under controlled room temperature (22±20°C) and humidity (55±5%). All the animals were provided with commercially available normal rat pellet diet and water *ad libitum*. Approval for the study protocol was granted by the Institutional Animal Ethics Committee of SBS (PG) Institute of Bio-medical Sciences and Research, Balawala, Dehradun, India (Reg. No. 273/CPCSEA).

Acute toxicity study[12]

Two groups of Albino mice of 10 animals per group and weighing 20-25 g were administered graded dose (100-2000 mg/kg body weight, orally) of the methanolic extracts of *Coccinia indica* (Wight & Arn) and *Salvadora oleoides* (Decne). After administration of the extracts animals were observed for general organ toxicity,

morphological behavior and mortality in any group for 7 days. No mortality was observed therefore the extracts were found safe for use up to the dose of 2000 mg/kg of body weight orally.

Antidiabetic Activity of Combined Methanolic Extracts (CMEt) [13]

Induction of diabetes

Alloxan monohydrate dissolved in distilled water was given intraperetoneally at a dose level of 150 mg/kg body weight to induce hyperglycemia in overnight fasted rats. 60 hours after the alloxan administration the rats were kept on fasting and were fed with water *ad libitum*. After 12 hours of fasting the animals were diagnosed for their blood glucose levels and those with serum glucose levels greater than 180 mg/dl were considered diabetic and selected for further studies.

Methanolic extracts of leaves of *Coccinia indica* and *Salvadora oleoides* were combined in a 1:1 ratio. The combined methanolic extract (CMEt) was suspended in 1% CMC and administered orally (once daily) at a dose of 150 mg/Kg body wt., continuously for 15 days. Glipizide was used as standard and was administered orally (once daily for 15 days) at a dose of 5 mg/Kg body wt., after suspending in 1% CMC.

Experimental Design

The rats were divided into 4 groups having 6 animals in each group as follows:

1. Normal Group I – received normal pellet diet and water.
2. Control Group II –received vehicle at a dose of 1 ml/kg b.w.
3. Group III (Combined Methanolic Extract (CMEt) – received CMEtin 1% CMCat a dose of 150 mg/kg b.w.
4. Group IV (Standard Drug): received Glipizide in 1% CMC at a dose of 5 mg/kg b.w.

Estimation of blood glucose, cholesterol and lipid profile, urea, creatinine, SGOT & SGPT activity

Blood glucose level was estimated on 1st, 5th, 10th and 15th day of the treatment. Blood glucose, total cholesterol, serum triglycerides, serum HDL, serum urea, creatinine, SGOT & SGPT estimation was done by using the Siemens diagnostic kits.

LDL cholesterol was calculated as[14]

LDL = Total Cholesterol - HDL - Triglycerides/5

VLDL was calculated using the formula[14]

VLDL = Triglycerides/5

Statistical Analysis

All results are expressed as mean±SEM and statistical difference was evaluated using one-way analysis of variance (ANOVA) followed by Newman-Keulsmultiple comparison test. Data were considered statistically significant at P value≤ 0.01. Statistical analysis was performed using Graph Pad Prism (ver. 5.01) statistical software.

RESULTS

Effect of CMEt on Serum Glucose Levels

Intra-peritoneal administration of alloxan monohydrate produced significant increase in serum glucose levels (305.3 ± 6.57 mg/dl) with respect to the normal control group (92.16 ± 2.58 mg/dl). Oral administration of CMEt at a dose of 150 mg/Kg body wt. (once daily), significantly ($p<0.01$) reduced the blood glucose level to 284.0 (4.79%), 214.7 (28.02%)& 148.8 (50.13%) mg/dl on 5th, 10th and 15th day of the treatment respectively. Glipizide at a dose of 5 mg/Kg body wt reduced the blood glucose level to 233.2 (23.34%), 167.8 (44.82%)& 113.5 (62.68%) mg/dl on 5th, 10th& 15th day of the treatment respectively(Table 1, Fig. 1).

Effect of CMEt on Lipid Profile

Alloxan induced diabetic rats showed significant increase in serum cholesterol (178.7 ± 4.97 Vs 81.26 ± 5.18 mg/dl in normal control rats), serum triglycerides (182.3 ± 3.55 Vs 101.1 ± 4.31 mg/dl in normal control rats), LDL cholesterol (124.9 ± 4.87 Vs 35.16 ± 5.22 mg/dl in normal control rats), VLDL cholesterol (36.46 ± 0.71 Vs 22.83 ± 0.91 mg/dl in normal control rats) as well as significant reduction in HDL levels (17.31 ± 0.85 Vs 25.88 ± 1.67 mg/dl in normal control rats). As shown in Table 2, administration of CMEt 150 mg/Kg reduced serum cholesterol, triglycerides, LDL, VLDL levels (101.9 ± 3.29, 113.5 ± 3.73, 54.96 ± 2.97, 22.7 ± 0.75) which was comparable to control group. The standard drug Glipizide 5 mg/kg also produced the same effect (126.9 ± 2.32, 130.1 ± 3.36, 79.23 ± 1.76, 26.01 ± 0.67 mg/dl respectively). Increase in HDL levels were more pronounced in animals treated with CMEt 150 mg/Kg and Glipizide 5 mg/kg (24.22 ± 0.96 and 21.68 ± 0.72 mg/dl) which was comparable (Fig.2) to control animals (17.31 ± 0.85 mg/dl). (Fig.2)

Effect of CMEt on renal and liver functions

Alloxan induced diabetic rats exhibited higher serum creatinine (1.68 ± 0.05 mg/dl) and urea (79.13 ± 1.96 mg/dl) levels as compared to those of normal control rats (0.89 ± 0.06 mg/dl and 38.84 ± 1.68 mg/dl) (Table 3). The SGOT and SGPT activity was also increased (60.17 ± 3.51 U/L and 66.83 ± 4.05 U/L) in diabetic rats as compared to normal control rats (22.83 ± 0.91 and 27.67 ± 1.12 U/L). Treatment with CMEt (150 mg/Kg) significantly reduced the elevated creatinine (Fig.3), urea (Fig. 4), SGOT & SGPT levels (Fig.5) (1.18 ± 0.05 mg/dl, 52.98 ± 1.49 mg/dl, 32.32 ± 1.62 U/L and 37.31 ± 2.61 U/L respectively), which was comparable to that of Glipizide treated animals. (1.082 ± 0.06 mg/dl, 34.91 ± 0.75 mg/dl, 24.05 ± 2.20 U/L, and 27.42 ± 3.19 U/L).

Table 1: Effect of Combined Methanolic Extract (CMEt) on serum glucose levels in Alloxan induced diabetic rats

Group	Serum Glucose Level (mg/dl) on different Days of treatment			
	Initial	5th Day	10th Day	15th Day
Group I – Normal Control	92.16 ± 2.58	91.5 ± 2.14 (0.72%)	93.1 ± 1.84 (-1.02%)	95.5 ± 1.67 (-3.62%)
Group II – Diabetic Control	305.3 ± 6.57	321.2 ± 6.02[a] (-5.19%)	336.2 ± 6.19[a] (-10.09%)	346.3 ± 6.65[a] (-13.43%)
Group III – Glipizide (5 mg/Kg)	304.2 ± 6.69	233.2 ± 4.88[b] (23.34%)	167.8 ± 4.16[b] (44.82%)	113.5 ± 2.87[b] (62.68%)
Group IV – CMEt (150 mg/Kg)	298.3 ± 13.02	284.0 ± 13.64[b] (4.79%)	214.7 ± 11.54[b] (28.02%)	148.8 ± 7.39[b] (50.13%)

All values are in mean ± SEM; No. of animals N = 6 for each group; [a] statistically significantly different from normal group ($p<0.01$); [b] statistically significantly different from diabetic control ($p<0.01$); Numbers in parenthesis show percentage reduction in blood glucose level as compared to Initial level.

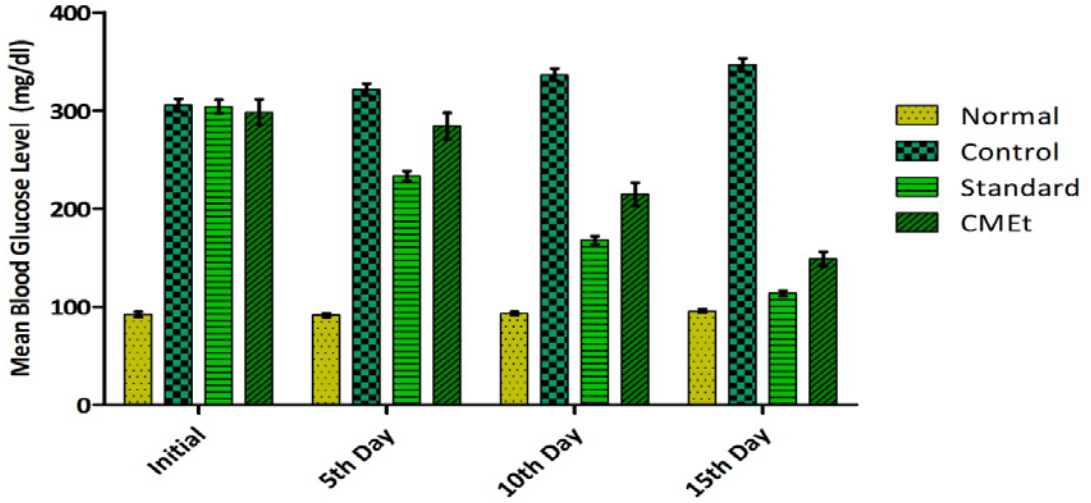

Fig. 1: Shows comparison of Serum glucose levels in all groups on different days of study (Small lines above each bar represent SEM)

Table 2: Effect of Combined Methanolic Extract (CMEt) on serum lipid profile Alloxan induced diabetic rats

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal Control	81.26 ± 5.18	101.1 ± 4.31	25.88 ± 1.67	35.16 ± 5.22	22.83 ± 0.91
Diabetic Control	178.7 ± 4.97**	182.3 ± 3.55**	17.31 ± 0.85*	124.9 ± 4.87**	36.46 ± 0.71*
Standard Glipizide (5 mg/Kg)	126.9 ± 2.32##	130.1 ± 3.36##	21.68 ± 0.72#	79.23 ± 1.76##	26.01 ± 0.67#
CMEt (150 mg/Kg)	101.9 ± 3.29##	113.5 ± 3.73##	24.22 ± 0.96#	54.96 ± 2.97##	22.7 ± 0.75#

All values are in mean ± SEM; No. of animals N = 6 for each group; *P<0.05, **P<0.01 significantly different from normal group; #P<0.05, ##P<0.01 significantly different from diabetic control;

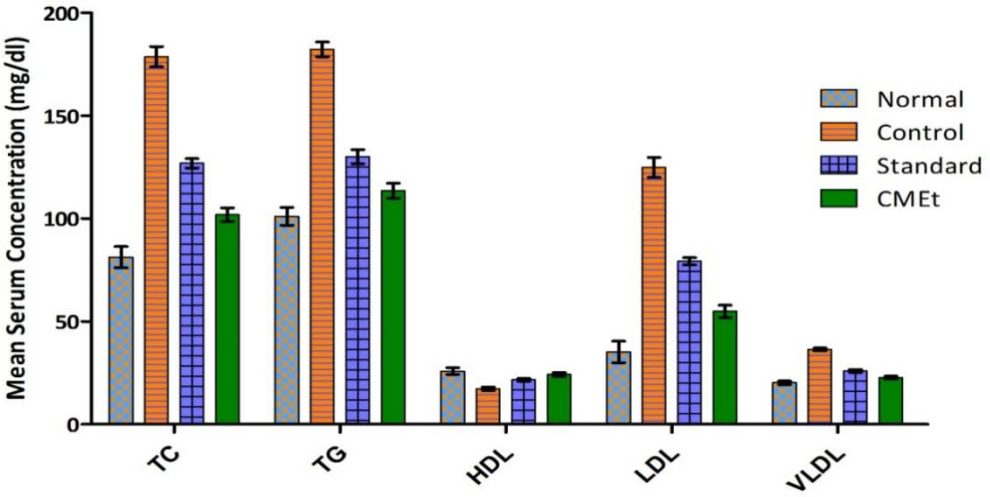

Fig. 2: Shows comparison of various parameters of Lipid Profile in different experimental groups (Small lines above each bar represent SEM)

Table 3: Effect of Combined Methanolic Extract (CMEt) on certain parameters of Liver Function & Kidney function in Alloxan induced diabetic rats

Groups	Creatinine (mg/dl)	Urea (mg/dl)	SGOT (U/L)	SGPT (U/L)
Normal Control	0.89 ± 0.06	38.84 ± 1.68	22.83 ± 0.91	27.67 ± 1.12
Diabetic Control	1.68 ± 0.05*	79.13 ± 1.96*	60.17 ± 3.51*	66.83 ± 4.05*
Standard Glipizide (5 mg/Kg)	1.082 ± 0.06**	34.91 ± 0.75**	24.05 ± 2.20**	27.42 ± 3.19**
CMEt (150 mg/Kg)	1.18 ± 0.05**	52.98 ± 1.49**	32.32 ± 1.62**	37.31 ± 2.61**

All values are in mean ± SEM; No. of animals N = 6 for each group; *P<0.01 significantly different from normal group; **P<0.01 significantly different from diabetic control;

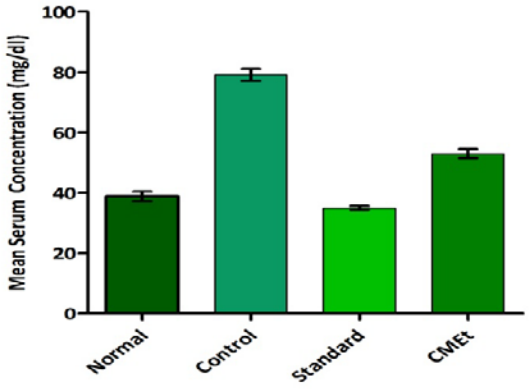

Fig. 3: Shows comparison of Serum Creatinine levels in various groups. (Small lines above each bar represent SEM)

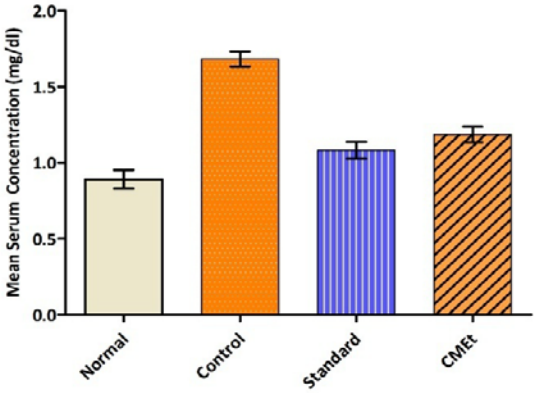

Fig. 4: Shows comparison of Serum Urea levels in various groups. (Small lines above each bar represent SEM)

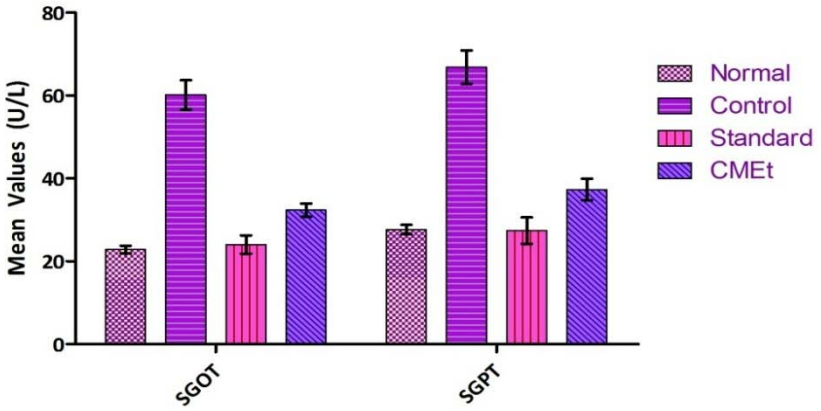

Fig. 5: Shows comparison of SGOT & SGPT levels in various groups. (Small lines above each bar represent SEM)

DISCUSSION

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, which produces inadequate glucose control and leads to acute and chronic complications. These complications increase the severity of the disease. Of these, cardiovascular disease causes most of the excess morbidity and mortality in diabetes mellitus. Cardiovascular disease accounts for up to 80% of premature excess mortality in diabetic patients[15]. Vascular complications can be caused by micro- and macro-angiopathy. Macro angiopathy in diabetes consists mainly of an accelerated form of atherosclerosis. Hyperglycemia plays an important role in the pathogenesis of microvascular complications.[16]Type 2 DM carries a high risk of large vessel atherosclerosis commonly associated with hypertension, Hyperlipidemia and obesity. Most patients with type 2 diabetes die from cardiovascular complications and end stage renal disease[17]. Natural products have played an important role throughout the world in treating and preventing human diseases[18].

The present study investigates the combined effect of methanolic extracts (CMEt) of leaves of *Coccinia indica* (Wight & Arn) and *Salvadora oleoides* (Decne) on blood glucose level and certain other markers of complications associated with diabetes including lipid profile, renal & liver functions, in alloxan induced diabetic rats.

Alloxan causes diabetes by pancreatic beta cell toxicity due to redox cycling and the generation of toxic reactive oxygen species.[19]Animals treated with CMEt (150 mg/Kg) for 15 days showed significant (P<0.01) reduction in serum glucose level (Table 1 & Fig. 1). The exact mechanism associated with the hypoglycaemic action cannot be stated. However some possibilities could be estimated based on the previous researches. Hypoglycemic action of *Coccinia indica* could be due to potentiating the insulin effect of plasma by increasing the pancreatic secretion of insulin from the existing β-cells.[20]*Coccinia indica* is also believed to bring about its antidiabetic action by stimulating glucose transport[9].The hypoglycaemic action of *Salvadora oleoides* could be associated with pancreatotrophic action.[10]

Diabetic rats were also observed to have increased plasma lipids, which are responsible for several cardiovascular disorders. Insulin deficiency may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key enzyme that is rate limiting in the metabolism of cholesterol rich LDL particles.[21]Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL-triglyceride from liver.[22] In diabetic rats, there is a decrease in lipoprotein lipase activity resulting in impaired clearance of VLDL and chylomicrons from plasma.[23]HDL cholesterol is inversely related to total body cholesterol and a reduction of plasma HDL cholesterol concentration may accelerate the development of atherosclerosis leading to ischaemic heart diseases, by impairing the clearing of cholesterol from the arterial wall.[24]

In our study, treatment with CMEt (150 mg/Kg) significantly (P < 0.01) decreased serum LDL, VLDL, Cholesterol and Triglycerides levels in diabetic rats as compared to the diabetic control group (Table 2, Fig. 2). It also produced significant increase (P < 0.01) in serum HDL level compared to diabetic control group. Probably CMEt leads to regeneration of the β-cells in the pancreas and increased insulin secretion from the surviving β-cells. This increase in insulin and consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. In this regard, some plants have also been reported to have antihyperglycemic, antihyperlipidemic and insulin stimulatory effects.[25, 26, 27]

There was also a significant increase in serum creatinine and urea levels in diabetic animals which indicate the impaired renal function. Treatment with CMEt significantly decreased the elevated serum creatinine and urea level in diabetic rats which was comparable to diabetic control group (Table 3, Fig. 3 & 4). This indicates the beneficial effect of the CMEt on renal function. Further investigation on few other markers of renal function may corroborate its efficacy. There are reports demonstrating increase in transaminase activities in liver and serum of diabetics. The increased level of transaminases which is active in absence of insulin because of availability of amino acid in blood of diabetics is responsible for the increased gluconeogenesis and ketogenesis observed in diabetics[28]. CMEt treated animals showed significant (P < 0.01) reduction in transaminase activity (SGOT & SGPT levels) compared to the diabetic control animals. (Table 3, Fig 5). This may indicate the revival of insulin secretion to normal levels; however this could be confirmed if insulin levels are estimated. The decrease in transaminase activity may also indicate the hepatic healing potential of CMEt which could be confirmed by detailed liver function test and histopathological studies.

Finally it could be concluded that the combined methanolic extracts (CMEt) of leaves of *Cocinia indica*(Wight & Arn) and *Salvadora oleoides*(Decne)possess a significant antidiabetic potential which is demonstrated by its effect on serum glucose level, lipid profile and renal and hepatic function. Various phytoconstituents like flavonoids, triterpenes, glycosides, alkaloids may be responsible for the observed activity. Further it could also prove beneficial in other risk factors associated with diabetes, especially hyperlipidemia which is of important concern in cardiovascular problems associated with diabetes. Moreover this could also lead to development of a cost effective natural medication for management of diabetes although further investigation is required to establishthe exact mechanism of antidiabetic action.

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