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Research Paper

EFFICACY OF METHANOLIC EXTRACT OF *Commiphora caudata* [WT. AND ARN.] ENG. BARK ON VARIOUS BLOOD PARAMETERS IN ALLOXAN INDUCED DIABETIC RATS

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Abstract

Crude Bark extract of *Commiphora caudata* [Wt. and Arn.] Eng. was prepared by soxhlet extraction method using methanol. Male albino rats of wistar staining weighing about 150-250 g were used in the present study. Diabetes was induced by single intraperitoneal injection of alloxan [150 mg/Kg]. The blood samples were drawn on 7th, 14th, 21st, and 28th day from the retrorbital venous plexus of rats under ether anesthesia using a glass capillary tube after a fast of 12 hrs and the blood was centrifuged [2,500 rpm/10 min] to get serum. The serum was used for biochemical estimation. On administration of MECCB [1 and 2 g/Kg, p.o, once daily] there was a significant decrease in the serum glucose levels on 7th, 14th, 21st and 28th day, when compared to diabetic control group [G-II]. Chronic treatment with methanolic extract of *Commiphora caudata* bark reduced blood glucose level throughout the experimental period in duration dependent manner indicating its anti-hyperglycemic activity. However blood glucose levels were not altered in normoglycemic rats further strengthening the hypoglycemia potential of the extract.

Key words: *Commiphora caudata* bark, Albino rats, Alloxan, hypoglycemia.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of carbohydrate, fat and protein metabolism. A defective or deficient insulin secretary response, which translates into impaired carbohydrate [glucose] use, is a characteristic feature of diabetes mellitus, as it results in hyperglycemia [Park K, 2005]. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications. This affects the function of various smooth muscles as a long-term complication. This chronic disease can cause various signs and complications like blindness, kidney failure and nerve damage and confer an increased risk of ischemic heart disease, stroke, amputation and peripheral vascular disease [James HO Keefe *et al.,* 2007]. Diabetes shortens life due to

micro and macro-vascular complications which are largely responsible for the morbidity and mortality associated with this disease. The higher the blood glucose levels and the longer the diabetic status, the more likely is the occurrence of complications [Alvin C. Power, 2005].

Oral hypoglycemic agents are useful in the treatment of diabetes mellitus but their use is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects. In addition to dietary and/or lifestyle modifications, oral antidiabetic agents and /or insulin can be used in diabetes. Presently available synthetic drugs have limiting factor of adverse drug reactions [ADRs] like hypoglycemia and weight gain [with sulfonylureas, thiazolidinediones], lacticacidosis [with biguanides] and oedema [with thiazolidinediones] etc. Recently, awareness of synthetic drugs related side effects have been increased tremendously and resulted in an increased demand of natural ingredients, as safe oral anti-diabetic agents [Rai PK *et al.,* 2010].

2. MATERIALS AND METHODS:

2.1. Collection of Plant Materials

The bark of *Commiphora caudata* [Wt. and Arn.] Eng. of family Burseraceae, commonly called Hill Mango collected from hill slopes of Tirumala, Andhra Pradesh, India and authenticated by Dr. Ravi Kiran, BSI, Coimbatore. Voucher specimens were deposited in Department of Botany, Acharya Nagarjuna University. Collected bark of *Commiphora caudata* was shade dried till the moisture content is evaporated and finally pulverized into small flakes.

2.2. Solvent Extraction

Crude plant extract was prepared by soxhlet extraction method. 100 g Commiphora *caudata* bark was uniformly packed separately into the thimble and extracted with 300 ml of methanol. Dried extract was kept in refrigerator at 4 ^{o}C for experimental study.

2.3. Animals

Male albino rats of wistar staining weighing about 150-250 g were used in the study. The animals were housed in group of 6 rats per cage and maintained under standard laboratory conditions at 24±2 ^{o}C in light controlled room [12 hrs dark and 12 hrs night] and provided commercial pellet diet. All the experimental protocols used for this study were reviewed by the Institutional Animal Ethics Committee with registration number CPCSEA\IAEC\EXP\25\50\2013\2013\EXP-04 and were in accordance with the guidelines of the CPCSEA.

2.4. Induction of Diabetes

In the present study, diabetes was induced by single intraperitoneal injection of alloxan [150 mg/Kg] [Katsumata K *et al.,*1992]. The alloxan was freshly prepared by dissolving 150 mg of alloxan in 1 ml of normal saline solution. The rats were allowed to drink 5 % glucose solution overnight to overcome the drug induced hypoglycemia.

2.5. Acute toxicity studies

Acute oral toxicity study was performed as per organization for Economic Cooperation and Development [OECD] guidelines 423. Stepwise dose of methanolic extract of *Commiphora caudata* [50 mg/Kg-2000 mg/Kg b.wt.], was administered. Animals were

observed individually during the first 30 minutes and periodically during the first 24 hrs, with special attention given during the first 4 hours and daily thereafter, for total of 14 days. The dose 2000 mg/Kg was found to be safe and no toxicity was observed. There were no toxic effects of mortality observed up to 14 days.

2.6. Selection of Dose

The LD50 cut off value found to be 2000 mg/ Kg. For evaluation of anti-diabetic activity two dose levels were selected i.e., first dose is one-tenth of LD50 cut off value and second dose was twice that off one-tenth dose [200 mg/Kg and 400 mg/Kg. p.o.single dose].

2.7. Experimental design

Table:1-Experimental Design

Group	Treatment
Group-I	Normal, untreated rats
Group-II	Diabetic Control rats
Group-III	Diabetic Control rats given Glibenclamide[0.5 mg/Kg of body weight]
Group-IV	Diabetic Control rats given MECCB [200 mg/Kg body weight]
Group-V	Diabetic Control rats given MECCB [400 mg/Kg body weight]

2.8. Collection of blood sample

The blood samples were drawn on 7^{th}, 14^{th}, 21^{st}, and 28^{th} day from the retrorbital venous plexus of rats under ether anesthesia using a glass capillary tube after a fast of 12 hrs and the blood was centrifuged [2,500 rpm/10 min] to get serum. The serum was used for biochemical estimation of blood glucose, triglycerides, cholesterol and Total serum protein.

3.RESULTS

Effect of methanolic extract of *Commiphora caudata* bark on serum glucose levels in alloxan induced diabetic rats is presented in the Table-2 and Fig-1. In the animals treated with alloxan [G-II] [150 mg/Kg, i.p, single dose] a significant increase in the serum glucose levels were observed on the 7^{th}, 14^{th} , 21^{st} and 28^{th} day, when compared to the normal animals [G-I]. This indicates that alloxan induced persistent diabetes mellitus.Group-III, that received standard drug [glibenclamide, 0.5 mg/Kg, p.o, once daily], there was significant decrease in the serum glucose levels on the 7^{th}, 14^{th}, 21^{st} and 28^{th} day, when compared to the diabetic control group. In standard group, the serum glucose levels approximately reached the normal on 21^{st} day. On administration of MECCB [1 and 2 g/Kg, p.o, once daily] there was a significant decrease in the serum glucose levels on 7^{th}, 14^{th}, 21^{st} and 28^{th} day, when compared to diabetic control group [G-II]. In both the groups [IV and V], the glucose levels were approximately equal to normal blood glucose levels on 21^{st} day. These results suggest the anti-diabetic activity of *Commiphora caudata* bark.

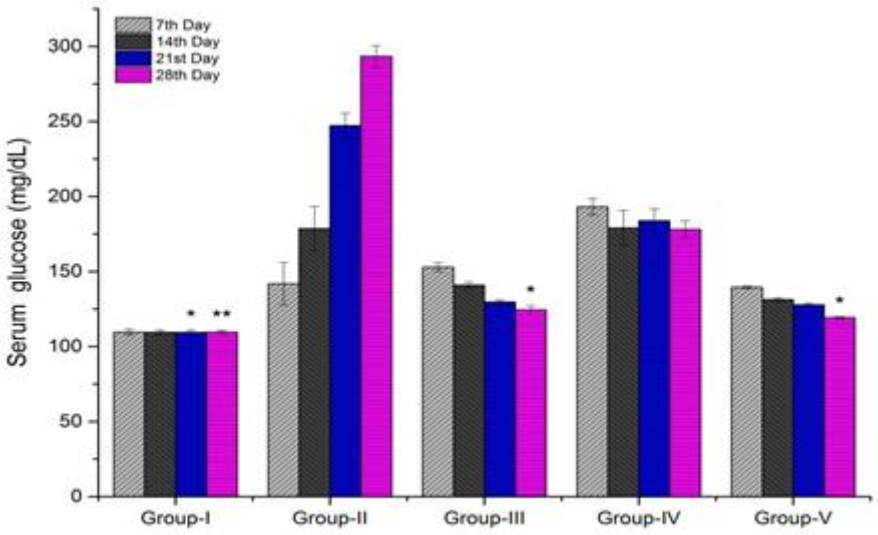

Fig-1: Effect of MECCB on Serum Glucose

Table-2: Effect of MECCB on Serum Glucose

Group	Treatment	Serum glucose [mg/dL]			
		7th Day	**14th Day**	**21st Day**	**28th Day**
Group -I	Normal	109.606±2.011	109.468±1.604	109.450±1.606 *	109.663±1.173 **
Group -II	Diabetic Control [150 mg/Kg. i. p]	141.870±14.201	178.605±14.767	247.255±8.258	293.368±7.174
Group -III	Glibenclamide [0.5 mg/Kg p. o.]	152.893±2.918	140.960±2.149	129.716±1.456	124.480±2.584 *
Group -IV	MECCB [200 mg/Kg p .o]	193.151±5.317	179.093±11.654	183.835±7.811	178.070±5.793
Group -V	MECCB [400 mg/Kg p .o]	139.495±1.015	131.365±1.094	127.811±1.142	119.293±0.610 *

The values were expressed as Mean ± S.E.M. [n=6 animals in each group]. Alloxan treated control [Diabetic control] was compared with the normal and extract treated, glibenclamide treated were compared with the alloxan treated control [Diabetic control].
***P < 0.001- Statistically highly significant; **P < 0.01 - Statistically very significant; *P < 0.05 - Statistically significant.

Effect of methanolic extract of *Commiphora caudata* bark on cholesterol levels in alloxan induced diabetic rats represented in the Table-3 and Fig-2. A significant increase in serum cholesterol level was observed in rats treated with alloxan [G-II] on 7th, 14th, 21st and 28th day, when compared to normal group [G-I]. The group-III rats treated with standard drug [glibenclamide, 0.5 mg/Kg, p.o, once daily] showed a significant decrease in serum cholesterol levels on 7th, 14th, 21st, and 28th day, when compared to the diabetic control group [G-II]. The groups [IV and V] receiving MECCB at a dose of 200 and 400 mg/Kg showed a significant decrease in serum cholesterol levels, when compared to diabetic control group [G-II]. The serum cholesterol levels were decreased

from 7th to 28th day in groups III, IV, and V. These values suggest that MECCB had cholesterol lowering activity.

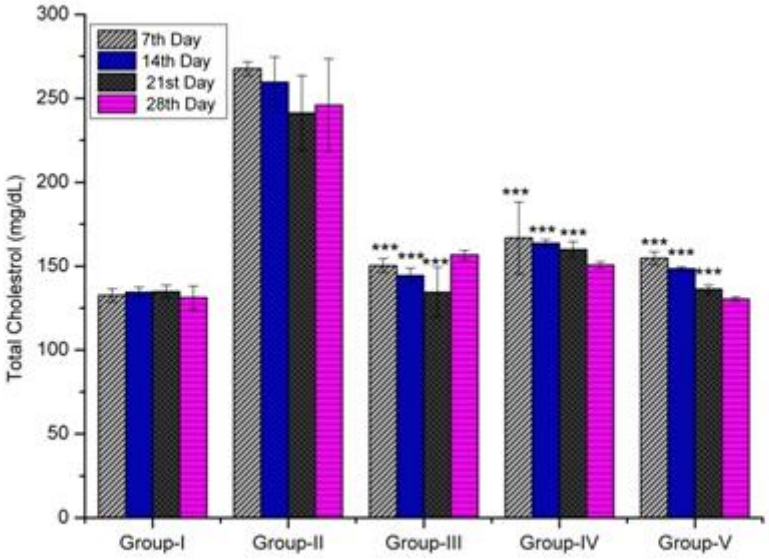

Fig-2: Effect of MECCB on Serum Cholesterol

Table-3: Effect of MECCB on Serum Cholesterol

Group	Treatment	Total cholesterol [mg/dL]			
		7th Day	**14th Day**	**21st Day**	**28th Day**
Group-I	Normal	132.575±3.895	134.358±3.173	134.838±3.751	131.183±6.997
Group-II	Diabetic Control [150 mg/Kg. i. p]	267.580±4.065	259.533±15.101	241.220±22.242	245.968±27.634
Group-III	Glibenclamide [0.5 mg/Kg p. o.]	150.306±4.154 ***	144.276±4.378 ****	134.475±14.890 ***	156.651±2.724
Group-IV	MECCB [200 mg/Kg p .o]	166.711±21.512 ***	163.555±2.274 ***	159.790±4.858 ***	150.576±2.048
Group-V	MECCB [400 mg/Kg p .o]	154.631±3.869***	148.375±1.243 ***	136.250±2.440 ***	130.336±1.457

The values were expressed as Mean ± S.E.M. [n=6 animals in each group]. Alloxan treated control [Diabetic control] was compared with the vehicle control and extract treated, glibenclamide treated were compared with the alloxan treated control [Diabetic control].

***P < 0.001- Statistically highly significant; **P < 0.01 - Statistically very significant; *P < 0.05 - Statistically significant.

Effect of methanolic extract of *Commiphora caudata* bark on triglyceride levels in alloxan induced diabetic rats illustrated in the Table-4 and Fig-3. Rats treated with alloxan [G-II] showed a significant increase in serum triglyceride levels on 7th, 14th, 21st and 28th day, when compared to normal group [G-I]. The group [III] rats treated with standard drug [glibenclamide, 0.5 mg/Kg, p.o, once daily] significantly lowered triglyceride levels on 7th, 14th, 21st, and 28th day, when compared to diabetic control [G-II].

Diabetic rats treated with MECCB [200 and 400 mg/Kg, p.o., once daily] showed a significant decrease in triglyceride levels on 7th, 14th , 21st and 28th day, when compared to the diabetic control [G-II]. These values suggest that glibenclamide and *Commiphora caudata bark* had triglyceride lowering activity and MECCB at high dose [400 mg/Kg] had exhibited similar effect on triglyceride as that of glibenclamide.

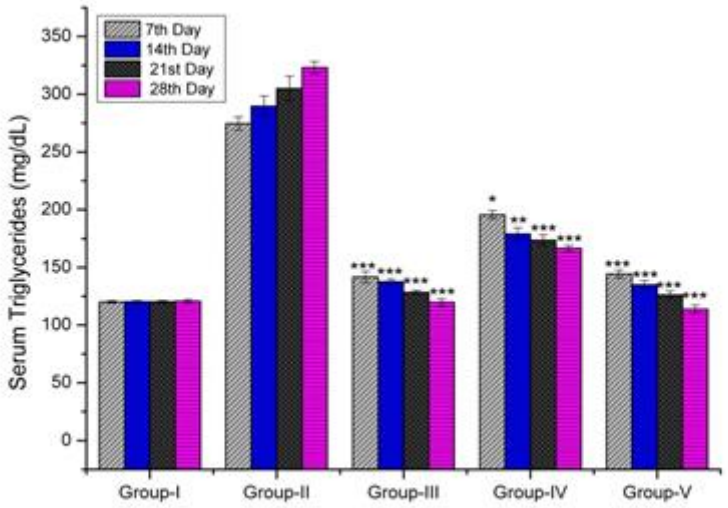

Fig-3: Effect of MECCB on Serum Triglycerides

Tables-4: Effect of MECCB on Serum Triglycerides

Group	Treatment	Serum Tiglycerides [mg/dL]			
		7th Day	14th Day	21st Day	28th Day
Group -I	Normal	120.183±1.031	120.263±1.037	120.268±1.092	120.761±1.562
Group -II	Diabetic Control [150 mg/Kg. i. p]	274.716±5.763	289.640±8.675	305.013±10.593	323.085±5.162
Group -III	Glibenclamide [0.5 mg/Kg p. o.]	141.658±4.918 ***	137.581±2.261 ***	128.448±1.716 ***	119.865±3.041 ***
Group -IV	MECCB [200 mg/Kg p .o]	195.593±3.648 *	178.848±5.488 **	173.466±4.709 ***	166.578±2.453 ***
Group -V	MECCB [400 mg/Kg p .o]	144.335±3.498 ***	134.928±3.613 ***	126.198±3.344 ***	113.750±3.309 ***

The values were expressed as Mean ± S.E.M. [n=6 animals in each group]. Alloxan treated control [Diabetic control] was compared with the vehicle control and extract treated, glibenclamide treated were compared with the alloxan treated control [Diabetic control].
***P < 0.001- Statistically highly significant;
**P < 0.01 - Statistically very significant;
*P < 0.05 - Statistically significant.

Effect of methanolic extract of *Commiphora caudata* bark on total protein levels in alloxan induced diabetic rats presented in the Table-5 and Fig-4. A significant increase in serum total protein was observed in rats treated with alloxan [G-II] on 7th, 14th, 21st and 28th day when compared to normal group [G-I]. The group-III rats treated with standard drug [glibenclamide, 0.5 mg/Kg. p.o once daily] showed a significant increase in serum total protein levels on 7th, 14th , 21st and 28th day when compared to the diabetic control group[G-II]. The groups [IV and V] receiving MECCB at a dose of 200 and 400 mg/Kg showed a significant increase in serum total protein levels, when compared to diabetic control group [G-II]. The serum total protein levels were increased from 7th to 28th day in groups III, IV and V.

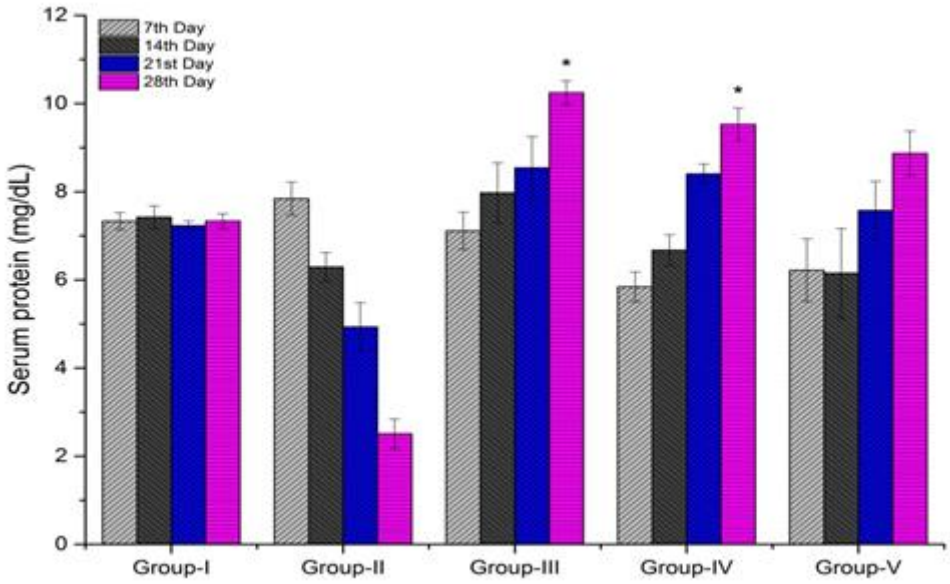

Fig-4: Effect of MECCB on Serum Total Protein

Table: 5-Effect of MECCB on Serum Total Protein

Group	Treatment	Serum Total protein			
		7th Day	**14th Day**	**21st Day**	**28th Day**
Group-I	Normal`	7.333±0.196	7.423±0.257	7.230±0.104	7.335±0.165
Group-II	Diabetic Control Alloxan [150mg/Kg. i. p]	7.845±0.377	6.293±0.330	4.931±0.553	2.508±0.337
Group-III	Glibenclamide [0.5 mg/Kg p. o.]	7.113±0.425	7.975±0.683	8.543±0.708	10.246±0.273 *
Group-IV	MECCB [200 mg/Kg p .o]	5.841±0.339	6.673±0.347	8.406±0.226	9.528±0.374 *
Group-V	MECCB [400 mg/Kg p .o]	6.220±0.713	6.153±1.016	7.576±0.660	8.870±0.509

The values expressed as Mean ± S.E.M. [n=6 animals in each group]. Alloxan treated control [Diabetic control] was compared with the vehicle control and extract treated, glibenclamide treated were compared with the alloxan treated control [Diabetic control].
***P < 0.001- Statistically highly significant;
**P < 0.01 - Statistically very significant;
*P < 0.05 - Statistically significant.

5.DISCUSSION

Alloxan-diabetic rats exhibited significant increase in blood glucose level. Chronic treatment with methanolic extract of *Commiphora caudata* bark reduced blood glucose level throughout the experimental period in duration dependent manner indicating its anti-hyperglycemic activity. However blood glucose levels were not altered in normoglycemic rats further strengthening the anti-diabetogenic potential of the extract.
Lipid abnormalities accompanying with atherosclerosis is the major cause of cardiovascular disease in diabetes. Therefore ideal treatment of diabetes, in addition to glycemia control, should have a favourable effect on lipid profiles. It was observed that there is an increase in the triglyceride and cholesterol levels in alloxan induced diabetic rats. This might have occurred in the diabetic rats as a result of lack of insulin which activates the lipase enzymes, hydrolyzing the stored triglycerides and releasing large amount of fatty acids and glycerol into the circulating blood. Consequently, the excess of fatty acids in the plasma may promote the hepatic conversion of fatty acids into phospholipids and cholesterol, the main products of lipid metabolism. At the same time glycogen, cortisol, catecholamine and growth hormones enhance lipolysis [Welt K *et al.,* 2007]. Hence, abnormalities in lipid metabolism can lead to elevation in the levels of serum lipid and lipoprotein that inturn play an important role in occurrence of premature and severe atherosclerosis, which affects patients with diabetes. Hence, measurements of lipid profiles are necessary to prevent cardiac complications in diabetic condition [Bainton D *et al.,* 1992].

Rats treated with MECCB showed significant reduction in total cholesterol and total triglyceride levels in alloxan induced diabetic rat model. These results suggested that MECCB has the potential role to prevent formation of atherosclerosis and coronary

heart disease. Several authors reported that secondary metabolites, such as saponins, flavonoids, phenolic compounds and triterpenoids, have hypolipidemic activity [EI-harzmi MA and Warsy AS., 2001]. Hence, the hypolipidemic properties of MECCB may be due to presence of flavonoids, phenolic componunds and saponins.

In diabetes mellitus, body cells are unable to utilize glucose as a source of energy due to which proteins are spared as energy source. This leads to decrease in protein storage which in turn reduces body weight [Guyton A C and Hall J E, 1996]. In the present study alloxan diabetic rats showed decrease in body weight throughout the experimental period. Oral treatment with methanolic extract of *Commiphora caudata* bark significantly improved the body weight loss in diabetic rats as compared to diabetic control indicating possible role of the extract in restoration of protein metabolism. The result of this present study therefore justifies the local use of
Commiphora caudata bark in the treatment of diabetes mellitus.

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