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Research Article

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Evaluation of Pharmacological activities of Siddha drug formulation Amirdhavalli Chooranam.

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Abstract

Siddha system of Medicine is a distinct therapeutic science with many single drugs and compound formulations used for treatinga broad spectrum of ailments. Amirdhavalli Chooranam(AVC)is a classical Siddha drug formulation used for the management and treatment of Madhumega noi (Diabetes Mellitus). This study is aimed to bring out the scientific validation for the therapeutic usage of AVC and it possesses on its pharmacological properties such as Anti-Diabetic, Anti-Oxidant, Hypolipidemic and Anti-Bacterial study have also been reported. These reports are very encouraging and indicate that the drug should be studied more extensively for its therapeutic benefits and is compiled to generate knowledge regarding its pharmacological activities controlling in Madhumega noi (Diabetes Mellitus).

Keywords: Amirdhavalli Chooranam, Anti- Diabetic, Anti-Oxidant, Hypolipidemic, Anti-Bacterial, Madhumega noi.

Introduction

Siddha medicine is one of the most ancient medical systems of India. Siddha medicines are known for its efficacy and safety. The reason for popularity of the Siddha system is attributed to its effectiveness with mimimal side effects. Herbal medicine has witnessed a renaissance among the customers throughout the world. The recent upsurge of interest in Siddha system of Indian medicine practiced in Southern parts of India can be seen by the large scale manufacture of Siddha formulation. However one of the impediments in the acceptance of the ancient systems of medical preparation is lack of standard quality control profile.

The test drug Amirdhavalli Chooranam mentioned in the classical Siddha text Gunapadam Mooligai vaguppu by Murugesa

mudhaliyar. The chooranam is prepared from the drug Seendhil(*Tinospora Cordifolia)* from Menispermaceae family.It is reported the medicinal properties like Anti-Diabetic, Anti-Oxidant, Hypolipidemic and Anti-Bacterial activity.

Materials and Methods

The whole plant of Seendhil were collected from in and around places of Palayamkottai. The raw drug is identified and authenticated by Medicinal Botanist of Government Siddha Medical College,Palayamkottai.All the parts of the plant of Seendhil were shade dried and ground into fine powder and filter to get the chooranam. The chooranam is stored in air tight container and labelled as Amirdhavalli Chooranam which was used for experimental purposes.

Anti-diabetic activity

Chemicals

STZ was obtained from S. D Fine. Chem. Ltd, Mumbai, India. Glibenclamide was obtained from Micro Labs, Hosur, India.

Selection and acclimatization of animals

Wistar strains of albino rats weighing between 180-200g are used for this study. The animals were housed in large spacious cages and they were fed with commercial pellets and access to water *ad libitum*. The animals were well acclimatized to the standard environmental condition of temperature (22 ± 5^0c) and humidity (55 ±5%) and 12 hr light dark cycles throughout the experimental period.

Induction of diabetes mellitus

Diabetes mellitus is induced in wistar rats by single intraperitoneal injection of freshly prepared solution of STZ monohydrate (150mg/kg Body Weight) in physiological saline after overnight fasting for 12hr

STZ is commonly used to produce diabetes mellitus in experimental animals due to its ability to destroy the β-cells of pancreas possibly by generating the excess reactive oxygen species such as H_2O_2, O_2. The development of hyperglycemia in rats is confirmed by plasma glucose estimation 72 hr post STZ injection. The rats with fasting plasma glucose level of 200-260mg/dl were used for this experiment.

Experimental procedure

In the experiment a total of 25 rats (20 diabetic surviving rats & 6 normal rats 5 in each group.) were used. Diabetes was induced in rats 3 days before starting the experiment. The rats were divided into 4 groups after the induction of STZ diabetes.

Treatment protocol

- **Group-I:** (Normal control) consist of normal rats treated with 10ml/Kg of normal saline, orally.
- **Group-II:** Diabetic control rats treated with 10ml/Kg of normal saline, orally.
- **Group-III:** (positive control) Diabetic rat received Glibenclamide (10mg/Kg) (kavalali et al.,2003) for 28 days, orally.
- **Group-IV:** (Treatment group) Diabetic rat received low dose 2 ml containing (200mg/Kg) AVC daily using intra-gastric tube for 28 days.
- **Group-V:** (Treatment group) Diabetic rat received high dose 2ml containing (400mg/Kg) of AVC daily using intra-gastric tube for 28 days.

Methodology

Sample collection:

After 28 days of treatment, the blood glucose level and body weight were measured. Then blood was collected from retro-orbital puncture under light ether anesthesia using capillary tubes. Blood was collected in fresh vials containing EDTA as anticoagulant agents and plasma was separated in a T8 electric centrifuge at 2000 rpm for 5 minutes. Then animal was sacrificed by euthanesia method. Liver and pancreas were

immediately dissected out, washed in ice-cold saline to remove the blood and liver was used for estimation of enzyme activity while pancreas was subjected to histopathological studies

Biochemical analysis

Estimation of blood glucose

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson Johnson based on glucose oxidase method.

Hepatic glucokinase and hexokinase activity

The part of liver for each test was perfused with ice cold 0.15M KCl and 1mM EDTA solution and homogenized twice its weight of ice cold buffer (0.01 cysteine and 1mM EDTA in 0.1 ml Tris-HCL, pH 7.4) and centrifuged for 20 min at 4^0C. Glucose phosphorylation was assayed by means of glucose 6 phosphate dependent spectrophotometric method.

Glucose-6-phosphatase activity

The part of the liver for each test was homogenized with 40 times its weight of ice cold buffer (0.1 citrate-KOH, pH 6.5) and filtered through cheese cloth. Glucose-6-phosphatase activity was measured by phosphate release by the method Marjorie. The determination of phosphoric acid concentration in assay mixture was done calorimetrically.

Glycogen Content

The tissue sample was digested by hot concentrated 30% KOH and treated with anthrone reagent. Glycogen content was determined calorimetrically.

Anti-oxidant activity:

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay.

The antioxidant activity of test drug sample AVC was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay . Sample AVC was mixed with 95% methanol to prepare the stock solution in required concentration. From the stock solution the serial dilution the concentration of 10,20,40,60,80,100,250,300 was made respectively. Ascorbic acid were used as standard was prepared in same concentration as that of the sample extract by using methanol as solvent. Final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample AVC at different concentration of 10,20,40,60,80,100,250,300 was noted after 15 min incubation period at 370C. Absorbance^{wasread} out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank.

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100.

The effective concentration of test sample AVC required to scavenge DPPH radical by 50% (IC50 value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

Hypolipidemic activity:

Animals

Wistar albino adult male rats weighing 150-200gm from animal housing facility of Vels University were housed in polypropylene cages maintained with temperature 27°C ±1°C and 12 hrs light and dark cycle. The animals were allowed to adapt to the environment for seven days and supplied with a standard pellet diet (Sai Durgafoods, Bangalore) and water *ad libitum*. The experimental protocol has got the approval IAEC bearing no AKCP/IAEC/76/20-21.

Chemicals

Atorvastatin obtained from local pharmacy, Tamil nadu (periyandavar medical). Diagnostic kits for estimation were purchased from Merck Diagnostics India Ltd. anesthetic ether, ethyl acetate, and ethanol (SD Fine Chemicals, Mumbai).

Atherogenic diet

Experimental hyperlipidemic diet: Experimental diet consists of well-pulverized mixture of cholesterol – 400 mg/kg, cholic acid – 50 mg/kg, and coconut oil. This mixture is made into paste-like molds and is fed to the rats.

Treatment with atherogenic diet

The prepared atherogenic diet was used in place of normal pellet diet to all the groups except control. Rats were exposed to atherogenic diet and water *ad libitum* for 20 days and were used to study the effect of AVC against experimental hyperlipidemia

Pharmacological Evaluation

All animals starved for 18 hours and provided water *ad libitum* before the experiment. The animals were divided into five groups of six rats each.

Group I served as normal control administered with 2% CMC only.

Group II served as hyperlipidemic control rats received atherogenic diet.

Group III and IV served as test groups received AVC 200mg/kg and AVC 400mg/kg respectively. Group V served as Atorvastatin (10mg/kg/day) considered as standard. All the groups except the normal control group administered received atherogenic diet After inducing the hyperlipidemia, the respective treatment was continued for 7 days. Animals were given standard pellet diet and water *adlibitum.*

Collection of blood

The next day after the completion of experimental study, the blood was taken from the rats under mild anesthetic state by retro orbital sinus puncture. The collected blood samples were centrifuged (2500 rpm) for 10 minutes. Then serum samples were separated and it was used for various biochemical analyses. Then animals were sacrificed and the liver, heart and kidney were taken for histopathological study.

Liver lipid extraction

The liver was homogenized in cold 0.15M KCl and extracted with CHCl3: CH3OH (2% *v/v*). This lipid extract was used for the estimation of lipid parameters.

Biochemical analysis

The serum and liver were analyzed for serum total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) by standard enzymatic calorimetric methods.

Anti-bacterial activity:

Sample Preparation

The given sample was dissolved in the concentration of 0.1g/1ml of Aqueous.

Test Organism:

The test microorganisms used for antimicrobial analysis *E.coli, Streptococcus mutans, Pseudomonas auroginosa and Staphylococcus aureus* were isolated from clinical samples and the bacterial strains were maintained on Nutrient Agar (NA).

Nutrient Broth Preparation

Pure culture from the plate were inoculated into Nutrient Agar plate and sub cultured at 37ºC for 24 h. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145

mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×108 cfu/ml. Standardized inoculum Used for Antimicrobial test.

Antibacterial Test:

The medium was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121^0C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture viz. analysis analysis *E.coli, Streptococcus mutans, Pseudomonas auroginosa and Staphylococcusaureus* Finally, About 10 µL of sample (Aqueous extract of AC) was loaded onto the disc then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity (Kohner *et al*., 1994; Mathabe *et al*., 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam *et al*., 2010)

Results

Anti-diabetic activity:

Statistical analysis

Results are expressed as mean±SEM. The treated groups were compared with control by ANOVA following Dunnett test. All the statistical analyses were carried out by using Graph pad Prism version 5.1 software.

Table 1 Effect of drug on body weight of normal and experimental animals in each group

Groups	Initial Body Weight (g)	Final Body Weight (g)
Group I (G 1)	**188.18 ± 0.56**	182.32 ± 0.56
Group II (G 2)	172.75 ± 0.08	148.57 ± 0.16*
Group III (G 3)	176.30 ± 0.76	193.33 ± 0.15*
Group IV (G 4)	193.78 ± 0.45	195.15 ± 0.76*
Group V (G 5)	183.29 ± 0.34	204.87 ± 0.23*

All values expressed as mean± SEM. * = p < 0.001, when compared to control. (G-I).

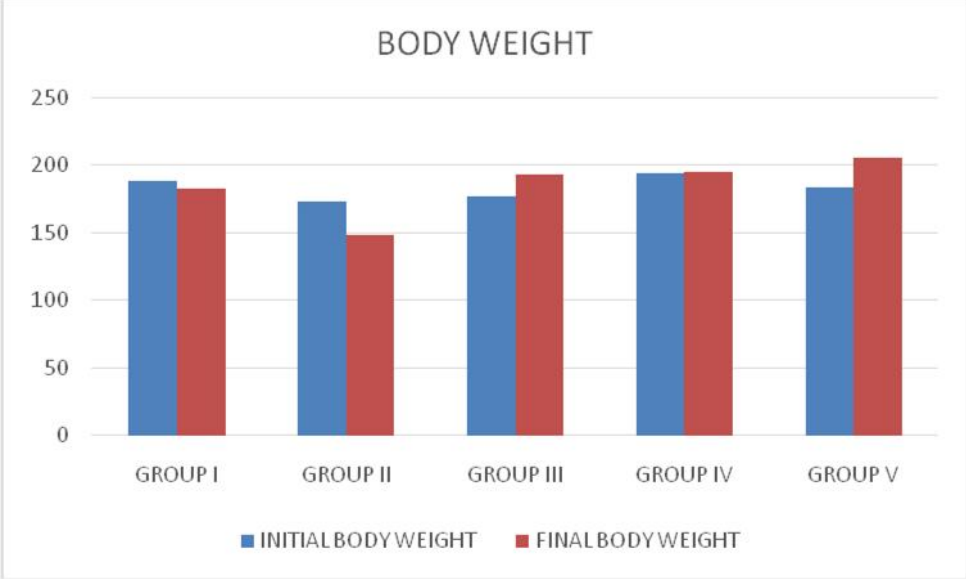


Fig.1:Total body weight in the diabetic-induced group have significantly decreased compared to normal rats. The values have risen to 195.06±0.25 mg/dl compared to Group I (normal rat group), in which values lie in the range 189.55 ± 0.75mg/dl. This indicates hypercholesterolemia. In the treatment group treated with PSP (200 mg/kg) and PSP (400 mg/kg), the values are reduced 193.68 ± 0.80($P < 0.001$) and 210.40 ± 0.85 mg/dl ($P < 0.01$), respectively. There is a significant reduction in total cholesterol values in 0TC treatment group. On the other hand, atorvastatin also has significantly reduced serum total cholesterol levels to 193.68 ± 0.80 mg/dl ($P < 0.001$)

Table 2 Effect of treatment (4 weeks) with various doses of drug on glucose levels (mg %) in STZ diabetic rats.

Groups	0Days		14th Day		28th Day	
	Before Meals	After Meals	Before Meals	After meals	Before Meals	After Meals
Group I	74.8±0.45	79.5 ± 0.13	69.16±0.16	72.26 ± 0.78	75.6±0.36	74.29 ± 0.98
Group II	85.34±0.57	141.30 ± 0.73	148.4±0.89	172.15 ± 0.89	102.1±0.56	202.6 ± 0.32
Group III	79.14±0.15	83.94 ± 0.37	80.41±0.20	81.56 ± 0.17	85.14±0.30	90.42 ± 0.12
Group IV	94.33±0.76	122.13 ± 0.32	136.72±0.11	152.25 ± 0.15	133.20±0.10	117.55 ± 0.10
Group V	114.85±0.16	119.20 ± 0.12	80.11±0.65	84.20 ± 0.76	127.75±0.19	112.60 ± 0.10

*** = p < 0.001, when compared to normal. (G-I), ** = p < 0.001, when compared to control. (G-II).**

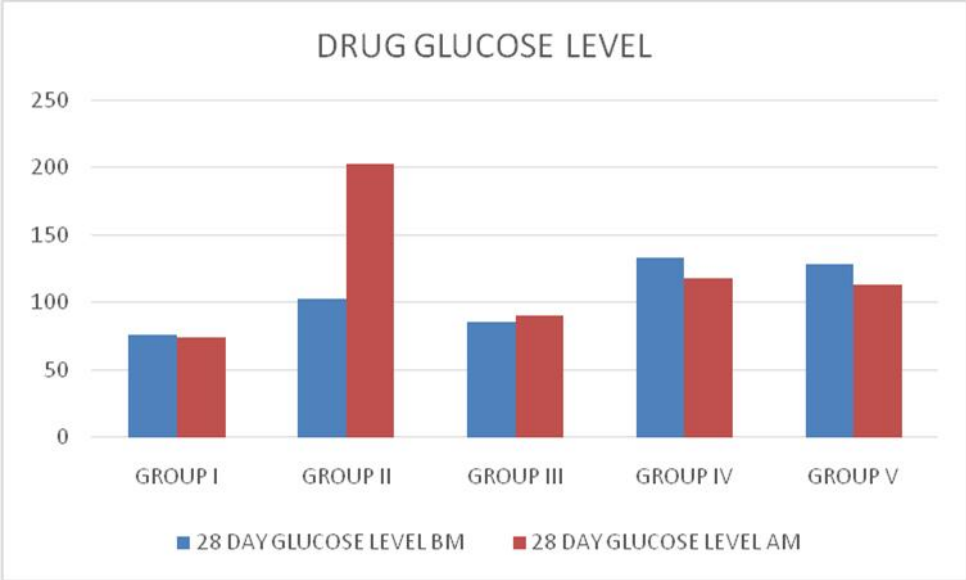


Fig.2:Serum glucose extensively raise in diabetic rats rate was observed on the 0, 14th and 28th day, once compared to the normal group (G-I). Group III given standard drug (0.5 mg/kg p.o glibenclamide) showed a serum glucose rates decrease considerably on the 7th, 14th, 21st, and 28th day, once compared to the diabetic control group (G-II). With the administration of AC in three different doses (G-IV and VI), were reduced blood glucose rates on the 0th, 14th, and 28th day, once compared to the control group (G-II) (Table 2)

Table 3 Effect of *drug* on glycogen content (mg/gm tissue)

Groups	Liver Tissue Glycogen Content (mg/g tissue)
Group I	36.45 ± 0.13
Group II	35.16 ± 0.74
Group III	28.50 ± 0.41
Group IV	19.10 ±0.66
Group V	20.45 ± 0.10

*** =p < 0.05, when compared to normal. (G-I), * =p < 0.01, when compared to control. (G-II), ** =p**

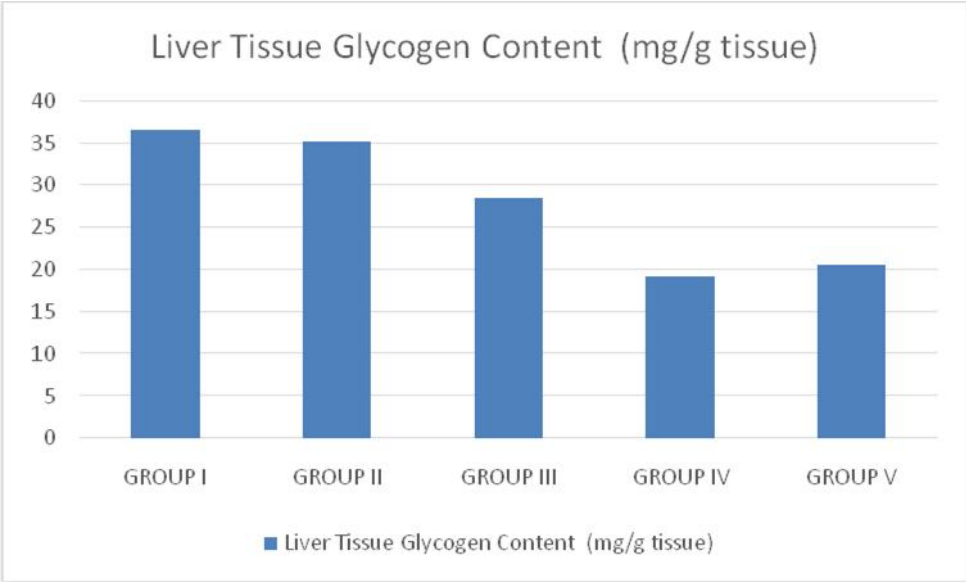


Fig.3:Effect of drug on Liver Glycogen Levels The diabetic control group (G-II) has shown a significant decrease in the rate of liver glycogen on the 28th day, once compared to the normal group (G-I). The hepatic glycogen has shown a significant rise in the standard group (G-III) which received glibenclamide, once compared to the diabetic control group (G-II). With the management of drug in three different doses (G-IV and G-V) on the 28th day, a significant increase in hepatic glycogen remained also noted, once compared to the diabetic control group (G-II) (Table 4).

Table 4 Effect of drug on enzymes involved in carbohydrate metabolism in rats

Groups	Hexokinase (µg/mg)	Glucose-6-Phosphate (µg/mg)	Glucokinase (µg/mg)
Group I	0.714 ± 0.16	0.808 ± 0.14	19.52 ± 0.15
Group II	0.156 ± 0.15	0.544 ± 0.239	8.19 ± 0.27
Group III	0.701 ± 0.29	0.797± 0.18	18.80 ± 0.16
Group IV	0.696 ± 0.24	0.668± 0.21	21.28 ± 0.16
Group V	0.706 ± 0.26	0.778 ± 0.38	19.27 ± 0.36

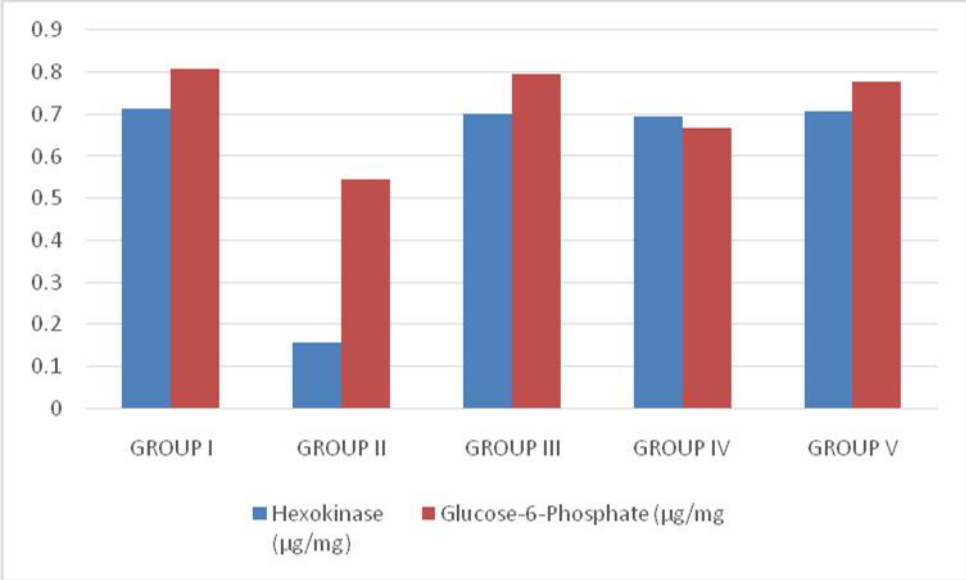


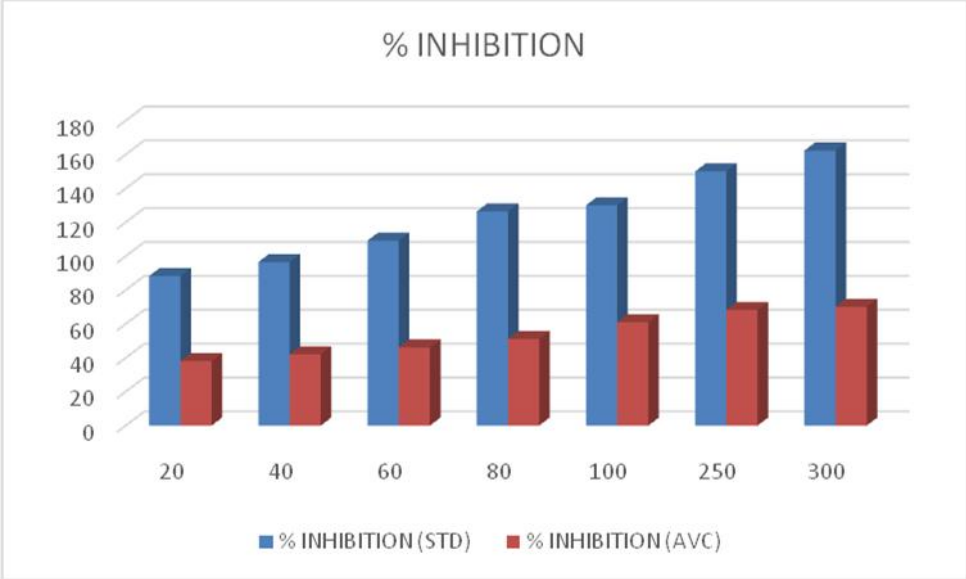
Fig.4:Effect of drug on Enzyme Levels The diabetic control group (G-II) has shown a significant decrease on the 28th day, once compared to the normal group (G-I). The enzyme has shown a significant rise in the standard group (G-III) which received glibenclamide, once compared to the diabetic control group (G-II). With the management of drug in three different doses (G-IV and GV,) on the 28th day, a significant increase in hepatic glycogen remained also noted, once compared to the diabetic control group (G-II) (Table 4).

Anti-oxidant activity:

Table.5:Result Analysis of DPPH radical scavenging Assay of AVC

S.No	Concentration	Ascorbic acid (Standard)		AVC	
		Absorbance	% inhibition	Absorbance	% inhibition
1	20	1.058±0.0017	88.29 %	1.020±0.008	38.06%
2	40	0.808±0.0015	96.50%	0.980±0.018	42.04%
3	60	0.680±0.0020	109.20%	0.870±0.022	46.10%
4	80	0.468±0.0025	126.40%	0.730±0.010	51.10%
5	100	0.273±0.0026	130.00%	0.620±0.012	61.01%
6	250	0.180±0.0028	150.05 %	0.542±0.029	68.14%
7	300	0.230±0.0031	162.30%	0.420±0.201	70.12%
	Ic 50 values		Ic_{50}= 6.1 µg/ml		Ic_{50} = 26.03µg/ml

The results of DPPH radical scavenging assay of the sample AVC shows that the test drug possesses concentration dependent scavenging activity on DPPH radicals.The value of DPPH free radical scavenging activity of the AVC was given in(Table 1 and Figure 1). The extract of AVC showed the highest DPPH scavenging activity 75.05% at conc 300 and the lowest percentage of inhibition (28.06 %) at conc 20 . Ascorbicacid (Standard) showed highest percentage of inhibition (162.30%) at 300 and the lowest percentage of inhibition 88.29 at conc 20.



Hypolipidemic activity:

Statistical evaluation

All the values were expressed as mean ± standard error of mean. The data were statistically analyzed by one-way ANOVA followed by Dennett's t-test, and value $P < 0.05$ was considered to be significant.

Table 6: Effect of *AVC* on body weight of atherogenic induced hyperlipidemic rats.

s.no	groups	Body weight
1	Normal control	149.34±0.49
2	Hyperlipiic Control	253.20±0.16
3	AVC (LOW)	176.59±0.37*
4	AVC (HIGH)	164.23±0.26*
5	Atorvasatin (10mg/kg/day)	153.75±0.63**

All the values were represented as mean±SEM. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's test and values *p<0.5* were considered to the significant.*p<0.001;**p<0.01 vs control,

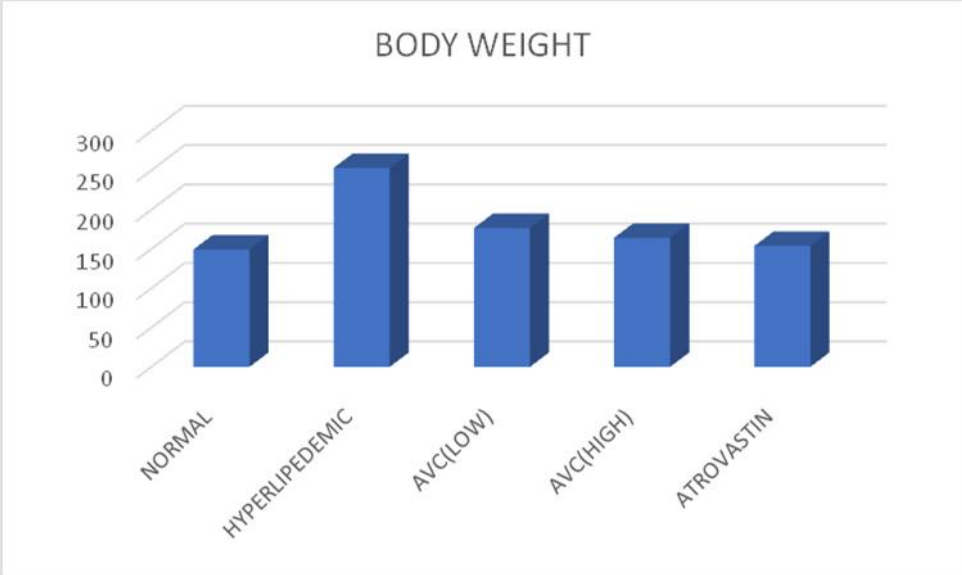


Fig.5:Total body weight in the hyperlipidemia-induced group have significantly increased compared to normal rats. The values have risen to 253.20±0.16 mg/dl compared to Group I (normal rat group), in which values lie in the range 149.34±0.49 mg/dl. This indicates hypercholesterolemia. In the treatment group treated with AVC (200 mg/kg) and AVC (400 mg/kg), the values are reduced 176.59±0.37 ($P < 0.001$) and 164.23±0.91mg/dl ($P < 0.01$), respectively. There is a significant reduction in total cholesterol values in AVC treatment group. On the other hand, atorvastatin also has significantly reduced serum total cholesterol levels to 153.75±0.63mg/dl ($P < 0.001$) [Table 6].

Table 7: Effect of *AVC on* Blood lipid profile of atherogenic -induced hyperlipidemic rats.

Group	Treatment	T.C.	T.G.	LDL	HDL	VLDL
I	Normal Control	73.15±0.17	73.25±0.15	20.23±0.46	40.19±0.18	13.16±0.16
II	Hyperlipiic Control	133.29±0.33	141.29±0.46	57.16±0.57	24.17±0.22	30.49±0.45
III	AVC (LOW)	92.46±0.14*	77.15±0.13*	37.69±0.28*	29.14±0.72*	19.54±0.44*
IV	AVC (HIGH)	93.25±0.344*	75.35±0.36*	31.43±0.16*	34.15±0.18*	17.14±0.19*
V	Atovastatin 10Mg/kg	72.6±0.23**	64.13±0.25**	20.23±0.39*	39.6±0.10*	15.39±0.51**

All the values were represented as mean±SEM. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's test and values *p<0.5* were considered to the significant.*p<0.001;**p<0.01 vs control,

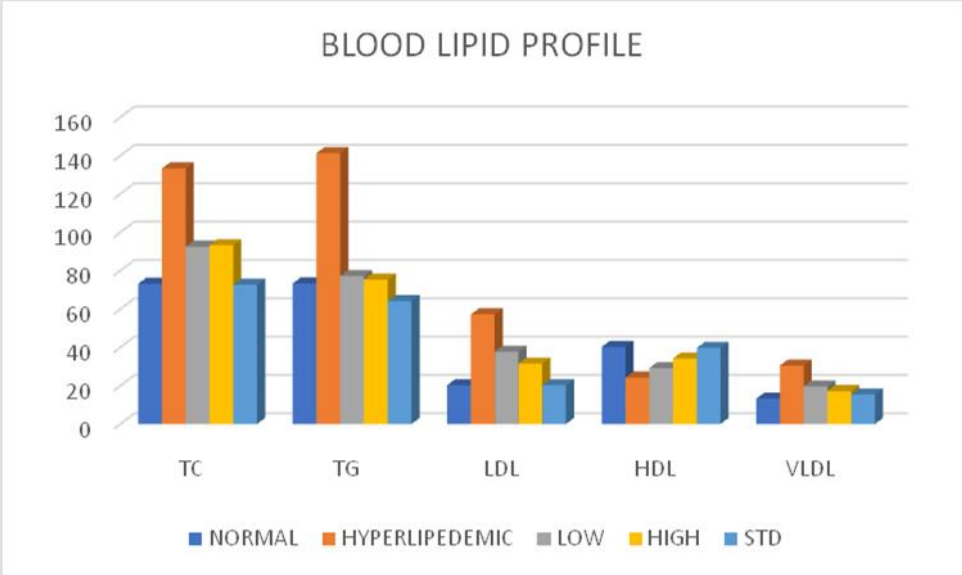


Fig.6:Total cholesterol levels in the hyperlipidemia-induced group have significantly increased compared to normal rats. The values have risen to 133.29 ± 0.33 mg/dl compared to Group I (normal rat group), in which values lie in the range 73.15 ± 0.17 mg/dl. This indicates hypercholesterolemia. In the treatment group treated with AVC (200 mg/kg) and AVC (400 mg/kg), the values are reduced to 92.46 ± 0.14 ($P < 0.001$) and 93.25 ±0.34 mg/dl ($P < 0.01$), respectively. There is a significant reduction in total cholesterol values in AVC treatment group. On the other hand, atorvastatin also has significantly reduced serum total cholesterol levels to 72.6± 0.23 mg/dl ($P < 0.001$) [Table 6].

The TG levels have reached as 141.29±0.46mg/dl in hyperlipedemia-induced group compared to normal rats where the values are 73.25±0.15mg/dl. This indicates triglyceridemia. In the group treated with AVC (200 mg/kg) and (400 mg/kg), the values are significantly reduced to 77.15±0.13*mg/dl ($P < 0.01$) and 75.35±0.36mg/dl ($P < 0.01$), respectively. In the atorvastatin treated group, the values are reduced to 64.13±0.25mg/dl ($P < 0.001$) [Table 6].

LDL-cholesterol in atherogenic-induced group has significantly increased to 20.23±0.46 mg/dl compared to normal rat group, 57.16±0.57mg/dl. In the group treated with AVC(200 mg/kg) and (400 mg/kg), the values were reduced to 37.69±0.28and 31.43±0.16 mg/dl ($P < 0.001$), respectively. There is a significant reduction in LDL-cholesterol values in AVC treatment group. atorvastatin has significantly reduced LDL-cholesterol level to 210.23±0.39mg/dl ($P < 0.001$) [Table 6].

HDL-cholesterol in atherogenic -induced group has significantly decreased compared to normal rats. The values have reduced to 24.17±0.22mg/dl compared to normal rat group, 40.19±0.18mg/dl. In the group treated with AVC(200 mg/kg) and (400 mg/kg), the values were 29.14.±0.72 ($P < 0.01$) and 37.17±0.18mg/dl ($P < 0.01$), respectively. In atorvastatin treated group, the values were 39.06±0.10mg/dl ($P < 0.001$) [Table 7].

VLDL-cholesterol in atherogenic-induced group has significantly increased to 30.49±0.45mg/dl compared to normal rat group, 13.46±0.16mg/dl. In the group treated with AVC (200 mg/kg) (400 mg/kg), the values are reduced to 19.54±0.44 ($P <$

0.01) and 17.14±0.19mg/dl (P < 0.01), respectively. There is a significant reduction in AVC treatment group. atorvastatin has significantly reduced VLDL-cholesterol level to 15.639±0.51 mg/dl (P < 0.001) [Tables 6].

Table 8: Effect of *AVC on* liver lipid profile of atherogenic -induced hyperlipidemic rats.

Group	Treatment	T.C	T.G.	LDL	HDL	VLDL
I	Normal Control	84.24±0.41	79.15±0.47	21.26±0.94	41.29±0.55	18.19±0.18
II	Hypolipidemic Control	165.76±0.22	167.89±0.30	50.15±0.16	26.15±0.17	40.27±0.47
III	AVC (LOW)	95.28±0.56*	93.17±0.08*	36.12±0.18*	30.20±0.40*	27.24±0.59*
IV	AVC (HIGH)	84.5±0.70*	83.12±0.33*	25.8±0.14*	39.10±0.14*	18.67±0.41*
V	Atorovastatin (10mg/kg/day)	82.34±0.13*	76.15±0.30*	22.72±0.15*	38.04±0.40*	17.38±0.15*

All the values were represented as mean±SEM. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's test and values $p<0.5$ were considered to the significant.*p<0.001;**p<0.01 vs control,

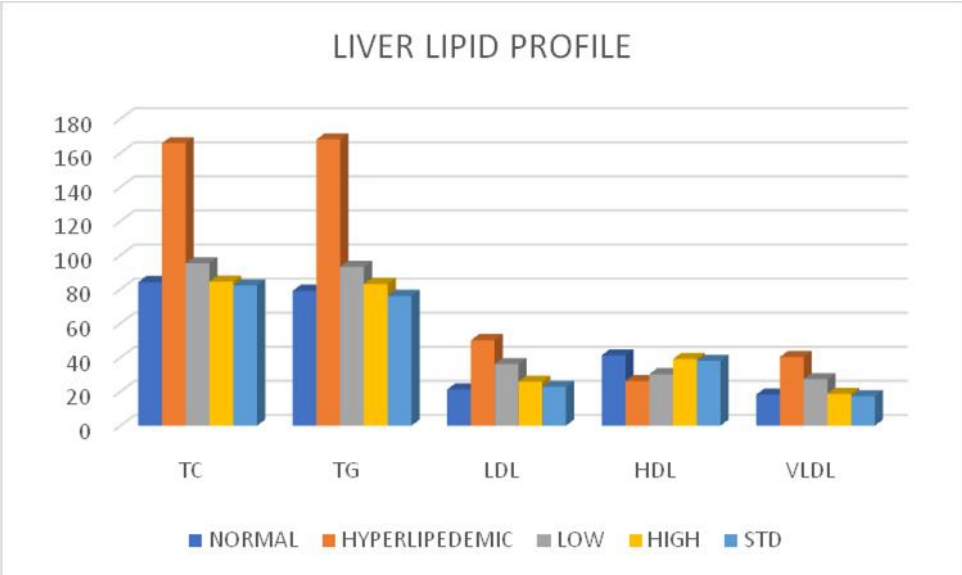


Fig.7:Total cholesterol levels in the hyperlipidemia-induced group have significantly increased compared to normal rats. The values have risen to 165.76±0.22mg/dl compared to Group I (normal rat group), in which values lie in the range 84.24±0.41mg/dl. This indicates hypercholesterolemia. In the treatment group treated

with AVC (200 mg/kg) and AVC(400 mg/kg), the values are reduced 95.28±0.56 ($P < 0.001$) and 84.5±0.70mg/dl ($P < 0.01$), respectively. There is a significant reduction in total cholesterol values in AVC treatment group. On the other hand, atorvastatin also has significantly reduced serum total cholesterol levels to 82.34±0.03 mg/dl ($P < 0.001$) [Table 6].

The TG levels have reached as 167.89±0.30 mg/dl in hyperlipedemia-induced group compared to normal rats where the values are 79.15±0.47 mg/dl. This indicates triglyceridemia. In the group treated with AVC (200 mg/kg) and (400 mg/kg), the values are significantly reduced to 93.17±0.08 mg/dl ($P < 0.01$) and 83.12±0.33mg/dl ($P < 0.01$), respectively. In the atorvastatin treated group, the values are reduced to 76.15±0.30 mg/dl ($P < 0.001$) [Table 6].

LDL-cholesterol in atherogenic-induced group has significantly increased to 50.15±0.16mg/dl compared to normal rat group, 21.26±0.94 mg/dl. In the group treated with AVC(200 mg/kg) and (400 mg/kg), the values were reduced to 36.12±0.18and 25.8±0.14mg/dl ($P < 0.001$), respectively. There is a significant reduction in LDL-cholesterol values in AVC treatment group. atorvastatin has significantly reduced LDL-cholesterol level to 22.72±0.15mg/dl ($P < 0.001$) [Table 6].

HDL-cholesterol in atherogenic -induced group has significantly decreased compared to normal rats. The values have reduced to 26.15±0.17mg/dl compared to normal rat group, 41.29±0.55mg/dl. In the group treated with AVC (200 mg/kg) and (400 mg/kg), the values were 30.20±0.40 ($P < 0.01$) and 39.10±0.14/dl ($P < 0.01$), respectively. In atorvastin treated group, the values were 38.04±0.40mg/dl ($P < 0.001$) [Table 8].

VLDL-cholesterol in atherogenic-induced group has significantly increased to 40.27±0.47mg/dl compared to normal rat group, 18.19±0.18 mg/dl. In the group treated with AVC (200 mg/kg) and (400 mg/kg), the values are reduced to 27.24±0.59 ($P < 0.01$) and 18.67±0.41 mg/dl ($P < 0.01$), respectively. There is a significant reduction in AVC treatment group. atorvastatin has significantly reduced VLDL-cholesterol level to 17.38±0.15 mg/dl ($P < 0.001$) [Tables 6].

Anti-bacterial activity:

Table: 9. Anti-bacterial potential of Aqueous extract of AVC

Sample Code and Conc.	**Bacteria Strains Name and Zone of inhibition (mm in diameter)**			
AVC	***Staphylococcus aureus* (G+)**	***Pseudomonas sps* (G-)**	***Streptococcus mutans* (G+)**	***E.coli* (G-)**
25	10	-	-	-
50	13	10	-	-
75	14	10	-	-
100	15	10	-	-
Positive Control (Streptomycin 25mg)	17	10	17	12
Negative Control	-	-	-	-

Keywords: ***PC*** *Positive control (Streptomycin),* ***NC*** *Negative control, "-" No Zone,* ***mm*** *(Millimetre),* ***G+*** *(Gram Positive Organism),****G-*** *(Gram Negative Organism),*

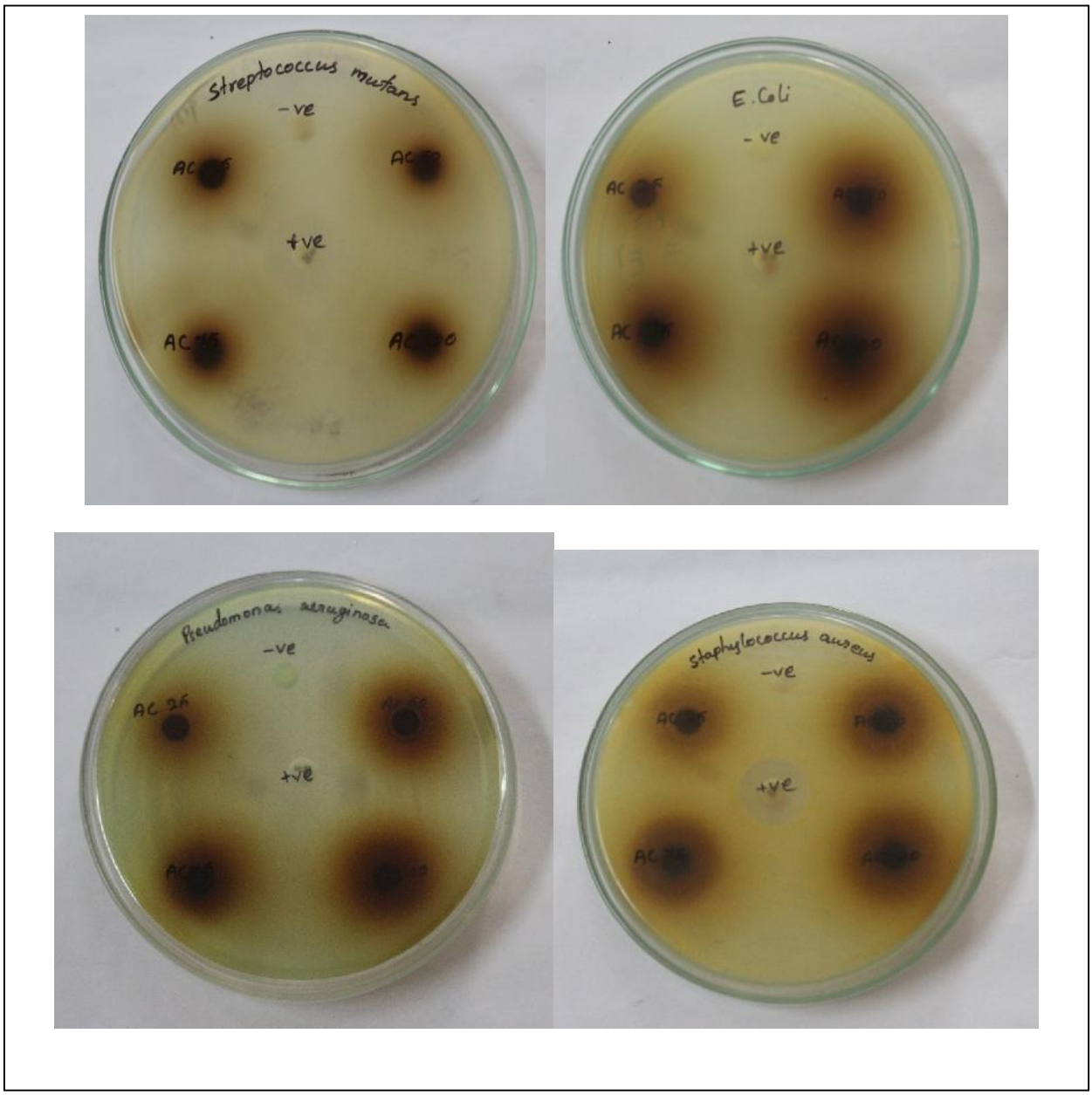


Figure: 7. Anti-bacterial potential of Aqueous extract of AC

Conclusion and Discussion

In conclusion, the drug at high dose (400mg/kg) and low dose (200mg/kg) exhibited significant anti-hyperglycemic activity in normal and STZ-diabetic rats. This powder showed improvement in the parameters like body weight, liver glycogen content and carbohydrate metabolizing enzymes as well as regeneration of β-cells of pancreas and so might be of value in diabetes treatment.From the result obtained from the present investigation it was concluded that the formulation AVC possess significant antioxidant property and may act therapeutically in treating several oxidative stress related disorder's. Further present investigation had generated an evidence based data with respect to purity, standards and antioxidant potential of the formulation AVC. The

results obtained from the pharmacological screening have led to the conclusions that, ac have significant antihyperlipidemic activity.

Hence it can be exploited as antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia. Further study by measurement of heparin-releasable plasma LDL activity and LCAT activity is significant can be undertaken. In Anti-Bacterial activity AVC has potential activity against Staphylococcus aureus and Pseudomonas aeruginosa.

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