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Original Article

ANTIDIABETIC AND HYPOLIPIDAEMIC ACTIVITY OF *CEIBA PENTANDRA*, *AMARANTHUS VIRIDIS* AND THEIR COMBINATION ON DEXAMETHASONE INDUCED DIABETIC SWISS ALBINO RATS

BUGGA PARAMESHA[1], VEMULA PRANAV KUMAR[1], RAMUDU BANKALA[1], K.MANASA[2], T.TAMILANBAN[1]*

[1]Bharath Institute of Technoogy, IbrahimPatnam, Hyderabad, Andhra Pradesh, India, [2]MNR College of pharmacy, Sangareddy, Hyderabad, Andhra Pradesh, India

Email: veera.srm@gmail.com

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ABSTRACT

Objective: We aimed to evaluate the antidiabetic and hypolipidaemic activity of ethanolic extracts of *Amaranthus viridis* and *Ceiba pentadra* and their combination on Dexamethasone induced Type-II diabetic Swiss albino rats.

Methods: Thirty animals were equally divided into the six groups as following: a control group (normal, non-diabetic), a diabetic group induced by subcutaneous injection of dexamethasone at a dose of 10 mg/kg for ten days. The standard drug treated group of animals were received metformin at a dose level of 10 mg/kg. Three test group animals were received plant extracts of *Amaranthus viridis, Ceiba pentandra* and their combination respectively at a dose levels of 400 mg/kg, 500 mg/kg and 450 mg/kg after simultaneous administration of dexamethasone subcutaneously for ten days. Body weight, blood glucose levels, serum lipid profile, liver and tissue glycogen levels were estimated.

Results: This study showed a significant increase in serum Tri-glycerides, Total cholesterol, LDL, VLDL and blood glucose levels and significant decrease in body weight, HDL, liver and tissue glycogen levels in diabetes control group when compared with normal group of animals. The treatment group showed significant improvement in all biochemical parameters.

Conclusion: Ethanolic extract of *Amaranthus viridis* and *Ceiba pentandra* and their combination showed a significant decrease in serum glucose, TG, TC, LDL, VLDL and significant increase in body weight, HDL, liver glycogen and tissue glycogen levels.

Keywords: Hypoglycaemic, Hypolipidemic, *Amaranthus viridis, Ceiba pentandra*, Dexamethasone.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia and increased lipid profile due to defect in carbohydrate, protein and fat metabolism [1]. Long term hyperglycaemic condition is associated with damage and failure of many organs such as eyes, kidney, nerves, heart, and blood vessels [2]. The defect in lipid, proteins and carbohydrate metabolism leads diabetic complications. Increased blood glucose levels modify the proteins such as elastin, collagen present in various tissues to glycoproteins responsible for the retinopathy, neuropathy, atherosclerosis and nephropathy [3]. Worldwide the occurrence of diabetes is estimated to progress, from 4 % in 1995 to 5.4 % by the year of 2025 [4]. The American Diabetes Association (ADA) criteria include signs of diabetes mellitus (e.g. polyuria, polydipsia and unexpected weight loss) and a random blood glucose level of more than 200 mg/dl, a fasting blood glucose level is more than 126 mg/dl or a blood glucose level of more than 200 mg/dl 2hours after administration of an oral glucose load [5]. Currently several therapies are used in the treatment of diabetes. Limitations of such therapies arehigh cost, hypoglycaemia, weight gain, gastrointestinal disturbance and liver toxicity are major distresses to search for alternative treatment to treat or control diabetes [6]. Naturally available compounds from plants are the other hand can provide an alternative treatment to treat diabetes [7].

Two major types of diabetes mellitus are

Type-1(IDDM): Insulin Dependent Diabetes Mellitus (juvenile onset diabetes mellitus). About 5-10 % children and young adult with age of 11-12 years has no ability to secretion of insulin because of destruction of pancreatic beta cells. Insulin dependent diabetes mellitus is an auto-immune disorder it occurs due to the destruction of pancreatic β- cells.

Type-2(NIDDM): Non-Insulin Dependent Diabetes Mellitus (maturity onset diabetes mellitus) about 80-90 % people over 40 years of age are more susceptible to type-2 diabetes. NIDDM occurs due to insulin resistance or impaired insulin secretion [8].

Ceiba pentandra

Ceiba pentandra belongs to Malvaceae family it is well known as silk cotton tree or local name known as dum. In African countries it is used as traditional medicine to treat several infections and disorders. Different morphological parts of plant are having different uses. It has been used treat hypertension, headache, dizziness, psychological disorders, fever, peptic ulcers, leprosy and diabetes. Folk medicine in Nigeria uses the root bark for the treatment of infections. In India and Malaya it is used for bowel complaints and it is used in the treatment of diarrhoea in West Africa [9].

Amaranthus viridis

Amaranthus viridis is well known cereal plant as well as leafy vegetable. It is well known due to its health benefits and easy availability [10]. Amaranthus is well known leafy crop, known for health welfare and easy availability [11]. *Amaranthus viridis* having high nutrition source of protein with proven medicinal values. In Goan folk medicine this plant is used as a tonic for weak patients to recovery [12]. Leaves of *Amaranthus viridis* are used as an emollient and used as remedy for snake bite. The roots are alone or in combination with the roots of *Cardiospermum hollicocardum* are used for scorpion sting. The aerial parts of the *Amaranthus viridis* with a pinch of the salt are used to cure constipation [12]. The aerial parts of the *Amaranthus viridis* have antidiabetic activity. Based on the findings and claims the above two plants are selected to evaluate the hypoglycaemic and hypolipidaemic activity of its individual and their combination effect on diabetes induced rats.

MATERIALS AND METHODS

Plant material collection

Amaranthus viridis aerial parts were collected in the month of November, 2012 in Uppal, Rangareddy district, AP and *Ceiba pentandra* bark was collected in the month of November, 2012 in Narsampet, Warangal district, AP and authenticated by Prof. Prathibha Devi, Head of department of botany, Osmania University, Hyderabad.

Preparation of extracts

The aerial parts of *Amaranthus viridis* were cut into pieces and *Ceiba pentandra* bark was cut into small pieces and shade dried at room temperature. The dried plant materials were subjected to size reduction to a course by dry grinder and passed through sieve no.40.

These powder materials were packed separately into soxhlet apparatus and extracted successively with ethanol as a solvent. Then the extracts of ethanol were concentrated. Extracts were dried by placing it on electric water bath at 70º C and then kept in an oven at 30º C for 2 hrs. The extracts obtained were stored in vacuum desiccators. The percentage yield of the *Amaranthus viridis* and *Ceiba pentandra* was 3.8 % and 4.6% respectively. The suspension of ethanolic extracts of both plant extracts were prepared by using 3.5% Tween-80 (SD fine chemicals, India) in normal saline solution [13].

Experimental animals

The antidiabetic and hypolipidaemic studies were carried out on albino WISTAR rats weighing 150-200gms. The animals were housed in the animal housed in the animal house of BIT and maintained in controlled temperature (27±2°C) and 12 hours light and 12 hours dark cycle. They were fed with rat feed and water add-libitum. The study protocol was observed and approved by institutional animal ethical committee of BIT (1015/C/06/CPCSEA, Dated on 5th may, 2009) [14].

Experimental design

The present study was carried out for 11 days to evaluate the effect of various treatments on biomarkers and glycogen content of liver and skeletal muscle tissues in dexamethasone induced insulin resistance in albino rats. The animals were randomly divided into six groups, five animals in each group. The animals were divided in to six groups as following.

Group- I: Normal control 2 ml/kg normal saline (vehicle) (p.o)

Group- II: Dexamethasone 10mg/kg (s.c) + vehicle (p.o) (Diabetic control vehicle)

Group- III: Dexamethasone 10mg/kg (s.c) + Metformin 5mg/kg (p.o)

Group- IV: Dexamethasone 10 mg/kg (s.c) + Ethanolic extract 500 mg/kg (p.o)

Group- V: Dexamethasone 10 mg/kg (s.c) + Ethanolic extract 400 mg/kg (p.o)

Group- VI: Combined plant extract 450mg/kg (p.o)

At the last day of the treatment schedule, i.e. on day 11, the overnight fasted animals were anaesthetized with diethyl ether and blood was collected by retroorbital puncture method. Separation of serum from collected sample by centrifugation at 5000 rpm for 5 minutes and stored at refrigeration temperature until use. Animal were sacrificed by cervical dislocation method to collected the tissue samples (Liver, skeletal muscle) and evaluated the tissue glycogen levels.

Phytochemical analysis

Phytochemical determination was performed as per standard text and presence of proteins, phenols, flavonoids, alkaloids, saponins, glycosides in *Ceiba pentandra* extract and fats, proteins, carotenes, alkaloids, glycosides, carbohydrates, flavonoids and sterols in *Amaranthus viridis* extract were determined [15,16].

Biochemical analysis

Blood glucose level, LDL, HDL, VLDL, TC, TG, liver glycogen, skeletal muscle glycogen content were evaluated. Fasting blood glucose level was measure by glucometer [17]. SeparatedSerum sample was used to estimate the serum cholesterol [18] and serum Tri glycerides by enzymatic DHBS colorimetric method [19], serum HDL [20], serum LDL, VLDL [21] and tissue glycogen was estimated [22].

Statistical analysis

All the data represented as Mean ± SEM were estimated by one-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test using prism graph pad version 5.0 and values of $p \leq 0.05$ were considered as statistically significant [23].

RESULTS

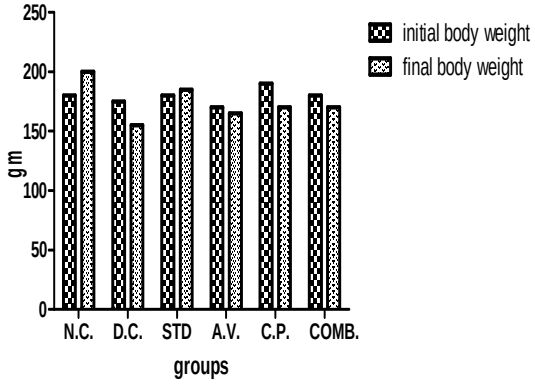

Fig. 1: It shows effect of different treatments on body weight of Dexamethasone induced diabetic rats:

The Body weight was decreased in type-2 diabetes induced by Dexamethasone. The plant extracts and standard drug treated groups had shown decreased reduction in body weight when compared to diabetic control group.

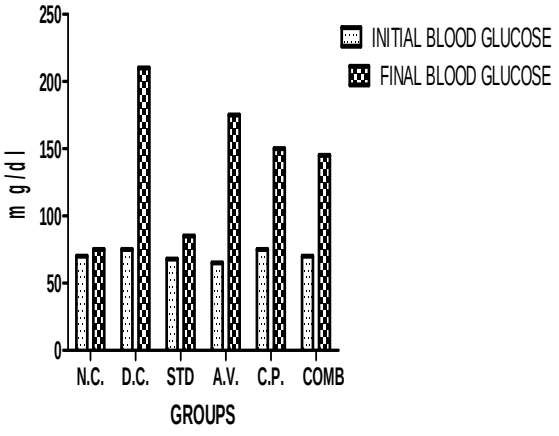

Fig. 2: It shows effect of different treatments on blood glucose level of Dexamethasone induced diabetic rats:

The Blood glucose level was increased in Dexamethasone induced diabetic rats. The plant extracts and standard drug treated groups had shown significant decrease in blood glucose level when compared to diabetic control group

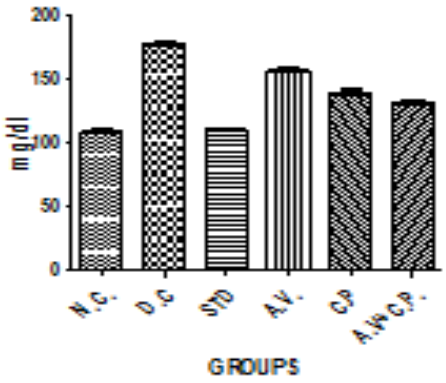

Fig. 3: It shows effect of different treatments on TG level of Dexamethasone induced diabetic rats:

The Serum Triglyceride level was increased in Dexamethasone induced diabetic rats. The plant extracts and standard drug treated groups had revealed significant decrease in triglyceride levels when compared to diabetic control group.

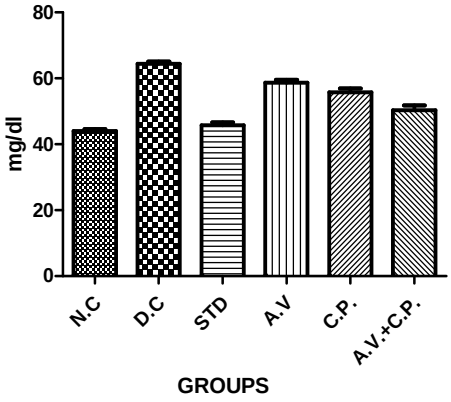

Fig. 4: It shows effect of different treatments on TC level of Dexamethasone induced diabetic rats:

The Serum Total cholesterol level was increased in Dexamethasone induced diabetic rats. The plant extracts and standard drug treated groups had shown significant reduction in the total cholesterol level when compared to diabetic control group.

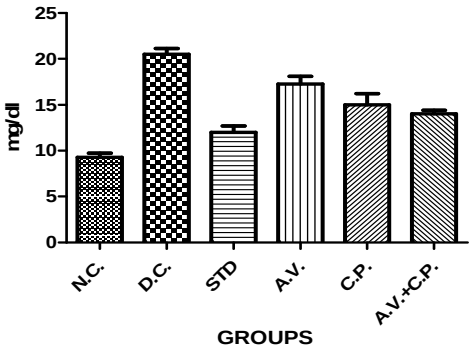

Fig. 5: It shows effect of different treatments on serum LDL level of Dexamethasone induced diabetic rats:

The Serum LDL level was amplified in Dexamethasone induced diabetic rats. The plant extracts and standard drug treated groups had shown substantial decrease in serum LDL level when compared to diabetic control group.

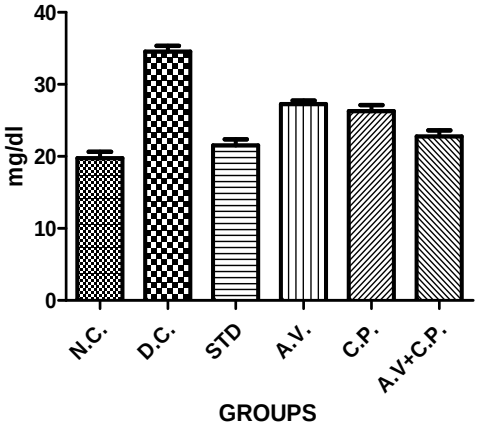

Fig. 6: It shows effect of different treatments on serum VLDL level of Dexamethasone induced diabetic rats:

The Serum VLDL level was amplified in Dexamethasone induced diabetic rats. The plant extracts and standard drug treated groups had shown substantial decrease in serum VLDL when compared to diabetic control group.

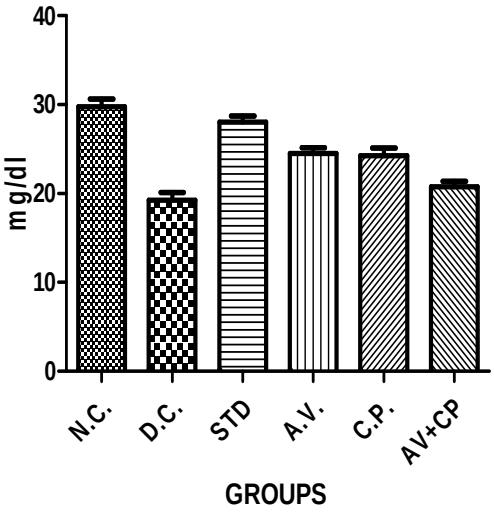

Fig.7: It shows effect of different treatments on serum HDL level of Dexamethasone induced diabetic rats:

The Serum HDL level was declined in Dexamethasone induced diabetic rats. The plant extracts and standard drug treated groups had shown substantial increase in serum HDL level when compared to diabetic control group.

The liver glycogen level was declined in Dexamethasone induced diabetic rats. The plant extracts and standard drug treated groups had shown substantial increase in liver glycogen level when compared to diabetic control group.

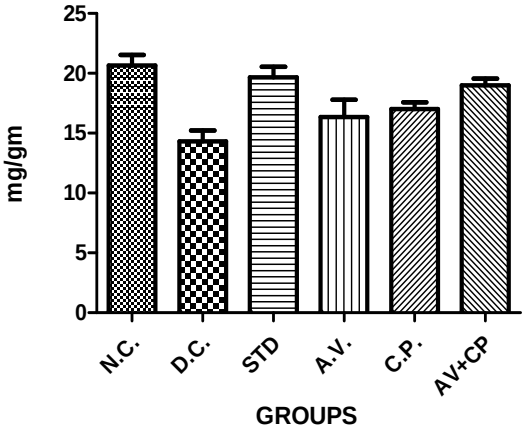

Fig. 8: It shows effect of different treatments on Liver glycogen level of Dexamethasone induced diabetic rats:

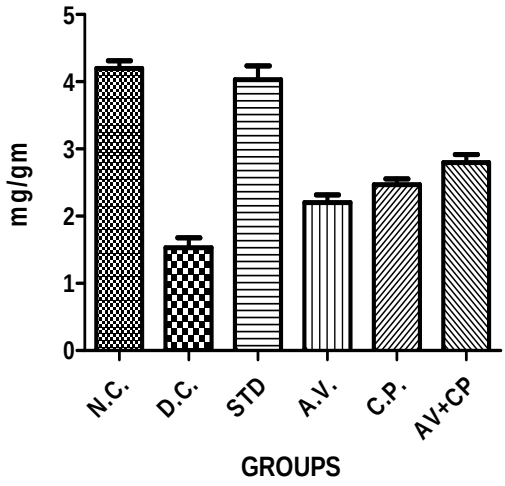

Fig. 9: It shows effect of different treatments on Tissue glycogen level of Dexamethasone induced diabetic rats:

The tissue glycogen level fell off in Dexamethasone induced diabetic rats. The plant extracts and standard drug treated groups had shown substantial increase in tissue glycogen level when compared to diabetic control group.

DISCUSSION

Non-insulin dependent diabetes mellitus or type-2diabetes mellitus is most predominant form of the diabetes mellitus. Type-2 diabetes mellitus occupied approximately 90 % of the diabetic patients and it causes serious socioeconomic problems in developing countries. Immunosuppressive drug glucocorticoid like dexamethasone at high dose level causes type-2 diabetes mellitus. Multiple numbers of high doses of dexamethasone represent insulin resistance by augmenting the insulin action on insulin dependent glucose uptake cells. Hepatocyte, skeletal muscle cells and adipose cells are insulin dependent cells. Insulin promotes the expression of GLUT-4 channels there by uptake of glucose increased by insulin dependent cells [24]. At high dose levels of dexamethasone causes protein break down and muscle wasting. Dexamethasone decreases the consumption of carbohydrates and lipids, decreases the glycogen synthesis and increases the protein break down.

Administration of dexamethasone at a dose of 10 mg/kg continuously for 10 days (DC) results in increase in serum glucose levels, LDL, VLDL, TG, TC decrease in body weight, HDL, liver and tissue glycogen content in diabetic control group rats. Administration of metformin and ethanolic extracts of *Ceiba pentandra*, *Amaranthus viridis* and its combination simultaneously with dexamethasone was showed significant decrease in serum glucose levels, LDL, VLDL, TC, TG and improvement in body weight, HDL, liver and tissue glycogen content when compared to diabetic control group of animals.

CONCLUSION

Results of present study discovered that the ethanolic extract of aerial parts of *Amaranthus viridis*, ethanolic extract of stem bark of *Ceiba pentandra* extract and its combination showed significant antihyperglycaemic activity and antihyperlipidemic activity but did not produce hypoglycaemic activity. It means that the ethanolic extract of *Amaranthus viridis* and *Ceiba pentandra* and its combination do not influence normal blood glucose levels of individuals. The mechanism of action of these plant extracts may be similar to that metformin. Both the standard and test drugs decreased the elevated blood glucose levels, LDL, VLDL, TC, TG and increases the HDL level, liver and tissue glycogen contents and decreased the reduction of body weight in diabetic rats. Results of this preclinical study give the necessary data for conducting phase-II clinical trials in type-2 diabetes. Finally further investigations are required to disclose the lead chemical constituent and its mechanism of antidiabetic activity.

ABBREVIATIONS

LDL- Low Density Lipoproteins; VLDL– Very Low Density Lipoproteins; HDL– High Density Lipoproteins; TG– Triglycerides; TC– Total Cholesterol; S.C– Subcutaneous; P.O – Per oral; DHBS- 3,5-dicholoro 2-hydroxy benzene sulfonic acid; AV– Amaranthus viridis; CP– Ceiba pentandra; NC– Normal Control; DC– Diabetic Control; STD– Standard

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CONFLICT OF INTEREST: Authors have no conflict of interest.

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