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Anti-Diabetic Hypolipidemic and Histopathological Analysis of Methanolic Leaf Extract of Celosia Argentea on Alloxan Induced Diabetic Rats

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ABSTRACT: To investigate antidiabetic, hypolipidemic and histopathological analysis of methanolic leaf extract of Celosia argentea in alloxan induced diabetic rats by administering oral doses (250 and 500 mg/kg body weight). Methods: During investigation the blood glucose levels were measured using blood glucose test strips with elegance glucometer on weekly intervals till the end of study (i.e. 3 weeks). On the other hand various parameters like liver profile, renal profile and total lipid levels were determined in normal and alloxan induced diabetic rats after oral administration of the extract for 21 days. After completion of study the effect of Celosia argentea on diabetic rat pancreas were also observed histopathologically. Results: Daily oral administration CAME (250 and 500 mg/kg body weight) and glibenclamide (10 mg/kg) showed beneficial effects on blood glucose level ($P<0.001$) as well as improving kidney, liver functions and hyperlipidaemia due to diabetes. The treatment with Celosia argentea also enhanced serum insulin level and body weight of diabetic rats as compared to diabetic control group. Furthermore, the Celosia argentea has a favourable effect on the histopathological changes of the pancreas in alloxan induced diabetes. Conclusions: The methanolic leaf extract of Celosia argentea possess antidiabetic property as well improve body weight, liver profile, renal profile and total lipid levels. Celosia argentea also has potential to inhibit the histopathological changes of the pancreas in alloxan induced diabetes

Keywords: Antidiabetic, Diabetes, Celosia argentea, hypoglycaemic.

I. INTRODUCTION

Diabetes mellitus (DM) is caused by inherited and or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. This insulin deficiency results in increased concentration of glucose in the blood. Hyperglycaemia induced because of two types of diabetes Type-I and Type-II. Out of these two types, Type –II diabetes is a major problem of today and it account for nearly 95% of total diabetic population of about 246 million[1]. In the pathogenesis of diabetes mellitus insulin level found to be reduced which results in hyperglycaemia which is managed by insulin and oral administration of hypoglycaemic drugs such as sulfonylureas and biguanides[2]. Every hypoglycaemic agent having a number of side effects, so there is no single oral synthetic hypoglycaemic agent has been successful in diabetes recovery and controlling long-term microvascular and macrovascular complications[2&4]. The best alternative for this is herbal medicines which are also preferred in the treatment of diabetes. The toxicity of oral hypoglycemic agent differs widely in severity and complications[5]. Herbal medicines have many advantages over oral synthetic hypoglycemic drugs like effectiveness, safety, affordability and acceptability [6]. In Indian traditional system of medicine number of herbal plants and their products have been used for prophylaxis of diabetes and has also proved as antidiabetic plants clinically [7, 8]. Evaluation of new herbal medicinal plant for prophylaxis of diabetes has also recommended by World Health Organization [9]. Since ancient times; plants have been an exemplary source of medicine. During rainy reason Celosia argentea grows as weed all over India and other tropical regions like Shri Lanka, South Asia, Africa and America. Traditionally it is used for prophylaxes of various disorders like jaundice, inflammation, fever and itching. Blood disorders and mouth sores treated

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with the help of seeds of Celosia argentea which have bitter taste. They are effective remedy for diarrhoea [1].Celosia argentea is leafy herb commonly known as quail grass, feather cockscomb, and Lagos spinach belongs to the family Amaranthaceae. The Celosia argentea contains a variety of species some of them includes spicata, cristata, c. argentea. This is also known as kurdu.This is the earliest classical herb in China, and is frequently used in traditional Chinese medicine for treating eye diseases, ulcer, to serve as anthelminthic, to treat trauma to blood, hygro-paralysis etc[10]. Celosia argentea leaves continually been used in Chinese medicine for the prevention and treatment of diabetes because it contains certain chemical compounds like flavonoids, alkaloids, polyphenols, terpenoids that suppress high blood sugar levels (hyperglycaemia) following a carbohydrate-rich meal. Based on recent research the Celosia argentea investigated for anti-inflammatory [11] anti – pyretic [12] anti diabetic [13], anti-bacterial and diuretic properties. On the basis of this and on account of alleged usefulness of Celosia argentea in the traditional treatment this current study was aimed to investigate anti-diabetic potential of methanolic extract of Celosia argentea leaves. For this purpose the methanolic leaf extract of Celosia argentea studied on alloxan induced diabetic rats to observe acute and chronic effects of extract on blood glucose levels of diabetic rats.

II. MATERIALS AND METHODS

2.1 Plant materials

The plant was collected from the fields located in outskirt of Bharati Vidyapeeth, morewadi, Kolhapur. Routine pharmacognostic studies were carried out to confirm identity of material. The Plant was authenticated by the Botany Department (Shivaji, University, Kolhapur, Maharashtra), Plant authentication voucher specimen number was (GGK-01).

2.2 Extract preparation

The leaves were shade dried and 1.5 kg coarsely powdered leaves were subjected to defattation using petroleum ether (60-80°C) in soxhlet apparatus and further same amount of material subjected to methanol for extraction. The extracts were concentrated and dried for further studies at reduced temperature and pressure in rotary evaporator. Yield obtained was 300 g (20%).

2.3 Chemicals

Alloxan was purchased from LobachemiePvt. Ltd. Mumbai, India. Total cholesterol (TC), serum high-density lipoprotein (HDL), serum creatinine (SC), serum urea (SU), serum alkaline phosphate (ALP), alanine transaminase (ALT), serum aspartate transaminase (AST) and triglyceride (TG) standard kits were obtained from Erma diagnostic kits, Japan. Blood glucose level was measured using accu-chek glucometer (Roche Diabetes Care, Mumbai). All reagents used in study were analytical grade.

2.4 Animals

Wistar rat of either sex, weighing about 150-250 g were purchased from Serum Institute of India Pvt. Ltd, Pune. Animals were maintained under standard environmental conditions i.e. ambient temperature of (22 ± 2) °C and at 45%-55% relative humidity for 12 h, each of dark and light cycle and fed with a standard pellet rats diet obtained from local market Gandhinagar, Kolhapur, India and water was supplied ad libitum. All the studies were conducted in accordance with the Animal Ethical Committee of the Bharati Vidyapeeth College of Pharmacy, Kolhapur. (Approval number BVCPK/CPCSEA/IAEC/01/09/2018-2019).

2.5Induction of diabetes

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (Am) (LobaChemie, Bombay; 150 mg/kg i.p.) in sterile saline. Twelve days after Alloxan injection, rats with blood glucose level of >200 mg/dL were separated and used for the study. Blood glucose levels were measured using blood glucose test strips with accu-chek glucometer (Roche Diabetes Care, Mumbai) at weekly intervals till the end of study (i.e. 3 weeks). Blood glucose estimation and body weight measurement were done on 0, 7, 14 and 21 day after administration of extract orally.

2.6 Experimental design

Overnight fasted rats were divided into five groups and for each group six animals and treated orally once a day for 21 days as follows:

Group I. Normal healthy control: given only vehicle (Tween 80, 1% v/v)

Group II. Alloxan (150mg/kg b.w.) and Vehicle (Tween 80, 1% v/v)

Group III. Alloxan (150mg/kg b.w.) and CAME-I (250 mg/kg b.w.)

Group IV. Alloxan (150mg/kg b.w.) and CAME-II (500 mg/kg b.w.)

Group V. Alloxan (150mg/kg b.w.) and Glibenclamide (10 mg/kg b.w.).

2.7 Biochemical parameters

Blood glucose level was estimated with accu-chek glucometer (Roche Diabetes Care, Mumbai) at weekly intervals i.e. 0, 7, 14 and 21

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days after daily oral administration of extract. After completion of study i.e. on the day 21 whole blood was collected from animals by cardiac puncture method using mild ether anaesthesia. In both normal and diabetic rats all biochemical parameters like Serum0 cholesterol, triglycerides, creatinine, urea, alkaline phosphatase, HDL and total proteins levels were evaluated[14].Serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were measured by autoanalyser (Erma Japan model PCI 210) using Erma diagnostic kits [15-16]. Serum insulin levels were determined using insulin ELISA kit [17].

2.8. Statistical analysis

Data ob`tained from this work were analysed statistically using Students t- test and ANOVA (One- or Two - way) followed by a post test (Tukeykramer multiple comparison test). Differences between means will be considered significant at 1% and 5% level of significance i.e P $\leq$ 0.01and 0.05.

III. RESULTS

3.1 Antidiabetic activity

Single dose of alloxan (150mg/kg b.w.) significantly (P<0.01) increases the blood glucose as shown Table 1. After the daily administration of Celosia argentea by oral route (250a and 500 mg/kg, p.o.) for 21 days significant decrease (P<0.01) in blood glucose levels was seen in alloxan induced diabetic rats. The orally administered Celosia argentea also significantly improved the reduced insulin level in diabetic rats The blood glucose level at the end of study i.e. on day 21 was (177.67±1.8 mg/dL) and (141.00±1.26 mg/dL) of the groups treated with the doses of Celosia argentea 250 and 500 mg/kg respectively (Table 1).

3.2. Effect on body weight of rats

Due to continuous administration of Celosia argentea by oral route for 21 days reduction in body weight was observed as shown in Table 2.Glibenclamide (10 mg/kg) as well as the extracts (Celosia argentea 250 and 500 mg/kg) treatment significantly (P<0.05) improved the body weight of diabetic rats.

3.3. Effect on lipid profile

The serum total cholesterol, triglycerides was significantly increased and HDL cholesterol was significantly decreased in alloxan induced diabetic rats as compared to normal control animals. The standard drug Glibenclamide as well as Celosia argentea (250 and 500 mg/kg) plant extracts used in the experiment significantly decreased (P<0.05) the levels of cholesterol and triglycerides whereas HDL cholesterol level was improved (Table 3) after 21 days treatment.

3.4 Effect on liver functions

In alloxan induced diabetic rats ALT, AST, ALP and bilirubin levels were significantly elevated. The continuous administration of Celosia argentea (250 and 500 mg/kg b.w.) showed significant (P<0.01) reduction in the raised levels of liver enzymes and the effect of Celosia argentea was induced in dose dependent manner. Also in alloxan induced diabetic rats the bilirubin level was decreased which was seen to be raised significantly after 21 days by treatment of Celosia argentea (250 and 500 mg/kg b.w.) (Table 4)

3.5. Effect on kidney functions

In alloxan induced diabetic rats the level of creatinine and urea were found to be raised which were significantly reduced by oral administration of Celosia argentea (250 and 500 mg/kg b.w.) and the effect of extract was in dose dependent manner. (Table 5)

3.6. Histology of pancreas

The figure no-1 illustrate the histological changes of rat pancreas of islets : (A) Normal control showing typical histological structure of rat pancreas (B) Diabetic control rat showing shrinkage of islets (C) Celosia argentea-I (250mg/kg) treated rat pancreas showing improved structure of islets (D) Celosia argentea-II(500mg/kg) treated rats shows significant results than diabetic groups and improved structure of islet (E) Glibenclamide (10 mg/kg) treated rats pancreas showing preserved islet architecture

IV. DISCUSSION

Alloxan monohydrate mainly leads destruction of β-cells of islets of langerhans so it acts as diabetogenic which induces hyperglycaemia by causing massive reduction in insulin release [18].Various metabolic alterations seen in alloxan induced diabetic rats like increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases etc[19-20].Administration of alloxan monohydrate (150mg/kg) as single dose by intra-peritoneal (i.p.) results in significant elevation in blood glucose levels of rats as shown in Table 1. At the end of study i.e. on day 21 this elevated blood glucose level were found to be reduced significantly by continuous oral administration of Celosia argentea and Glibenclamide. The insulin level has also raised by administration of Celosia argentea. So it was proved that the antidiabetic potential of

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Celosia argentea may be due to stimulation of insulin secretion. The treatment with Celosia argentea also significantly improved the weight loss which was observed in diabetic rats by restoring the urinary glucose and protein release. Hypertriglyceridemia and hypercholesterolemia are the common lipid abnormalities in diabetes [21]. Continuous administration of Celosia argentea for 21 days significantly ($P<0.05$) reduced hypertriglyceridemia and hypercholesterolemia this effect of Celosia argentea was may be due to inhibition of cholesterologenesis and fatty acid synthesis [22-32]. The Celosia argenteasignificantly improved HDL cholesterol level. Due to liver damage the level of liver enzymes like AST, ALT and ALP was found to be increased in diabetic rats and this were significantly reduced by Celosia argentea treatment. In diabetic rats the serum urea and creatinine were increased which were significantly improved by Celosia argentea. In case of alloxan induced diabetes the free radical production was found to be increased which results in tissue injury. Alloxan has been shown to induce free radical production and cause tissue injury. For this analysis the histopathology of pancreas were undertaken in which it was found that Celosia argentea was non-toxic and it reverts the toxic effects of alloxan. The leaves of Celosia argentea are rich in flavonoids which were proved from UV spectroscopic analysis. Flavonoids are antioxidant in nature so this antioxidant potential of leaves induce protective effect on the pancreas. So the result of present experiment showed that Celosia argentea reduces increased blood glucose level and improved body weight to normal in alloxan induced diabetic rats. It also improved kidney, liver function and hyperlipidaemia due to diabetes. In histopathological analysis the treatment of Celosia argentea was found to be favourable by producing protective effect on pancreas of alloxan induced diabetic rats. Therefore the antidiabetic potential of Celosia argentea leaves in diabetic rats may be possible through the insulinomimetic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production, or activation of gluconeogenesis in liver and muscle. Although the exact mechanism responsible for the hypoglycemic effects of Celosia argentea still remain speculative, experimental evidence obtained from this study indicates that Celosia argentea leaves possess antidiabetic property, which also is confirmed by histopathologic studies.

Conflict of interest statement

The authors report no conflicts of interest in this work.

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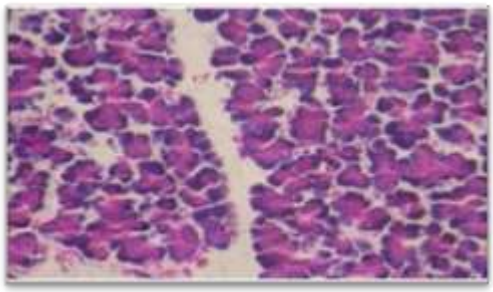
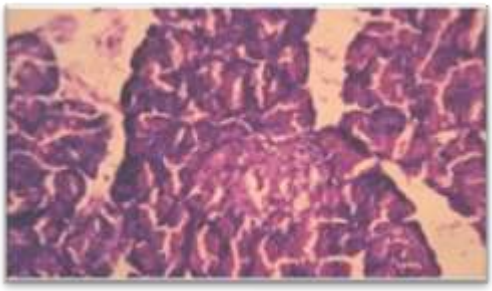
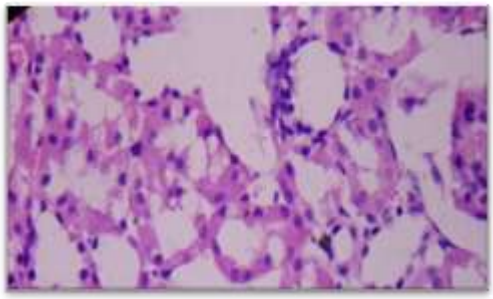


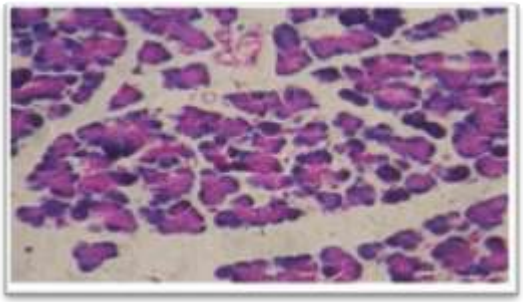
(A) Normal



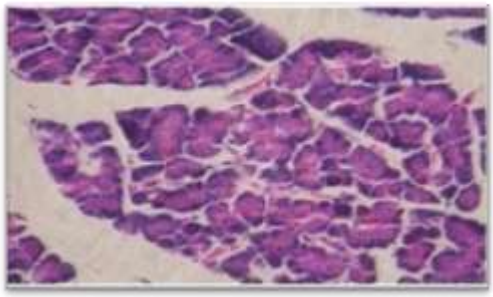
(B) Diabetic Control



(C) Celosia argentea-I



(D) Celosia argentea-II



(E) Glebenclamide

Figure No.1Effect of CAME on rat pancreas (A: Normal rats, B: Diabetic rats, C: Celosia argentea 250mg/kg, D: Celosia argentea 500 mg/kg and E: Glebenclamide 10mg/kg).

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Table 1

Effect of Celosia argentea on the blood glucose and insulin levels in alloxan diabetic rat (A-D) (n=6).

DRUG	DOSE (mg/kg)	BLOOD GLUCOSE LEVEL(mg/dL(Mean + SD)				SERUM GLUCOSE LEVEL(IU /dL)(
		INITIAL	7th day	14th day	21th day	
Normal		111.33±1.21	111.00±1.41	113.17±1.25	112.00±1.30	4.54±0.10
Control	-	225.50±1.97	272.83±1.72	284.83±1.33	310.67±1.51	1.30±0.03
CAME-I	250	229.17±1.72	219.67±1.03	199.33±1.33	177.67±1.86	2.28±0.04
CAME-II	500	229.33±1.03	203.33±1.37	176.00±1.41	141.00±1.26	2.93±0.35
GBL	10	225.67±1.37*	179.17±1.47	150.33±1.03	115.33±1.37	3.48±0.15

Data represent means ± S.E.M. *P<0.05, **P<0.01, When groups III, IV and V compared with diabetic control i.e. group II, N= Numbers of animals in each group.

Table 2

Effect of Celosia argentea on the body weight in diabetic rats (n=6). Groups/Treatments Body weight (g) Initial day

DRUG	DOSE (mg/kg)	Body weight in diabetic rats (G) (Mean + SD)				Total weight gain (G)
		Initial day	7th day	14th day	21th day	
Normal		198.17±1.60	202.67±2.66	211.33±1.51	217.67±2.58	19.50
Control	-	200.33±2.42	213.17±2.14	227.50±2.59	245.00±2.10	44.67
CAME-I	250	200.17±1.17	207.33±1.21	217.67±2.16	232.67±1.97	32.50
CAME-II	500	200.17±1.17	206.50±1.05	215.67±1.63	225.33±1.63	25.17
GBL	10	201.17±1.94	206.67±1.37	213.83±1.83	222.67±3.01	21.50

Table 3

Effect of Celosia argentea on lipid profile (mg/DL) in alloxan induced diabetic rats

Groups/Treatments	Total cholesterol	Triglycerides	HDL cholesterol
Normal	88.50±1.38	83.33±1.21	46.99 ±1.26
Control	258.50±1.22	152.83±1.60	33.72 ± 0.6
CAME-I	120.00±1.79	115.50±1.38	47.11 ± 0.61
CAME-II	100.67±1.21	91.83±1.17	53.37 ±1.24
GBL	95.17±1.47	82.00±1.26	58.52 ± 1.54

Data represent means 依 S.E.M. *P<0.05, **P<0.01.

Table 4

Effect of Celosia argentea on liver parameters in normal and diabetic rats.

Groups/ Treatments	DOSE (mg/kg)	Total protein(g/dL)	Bilirubin(mg/dL)	AST(U/L)	ALT(U/L)	ALP(U/L)
Normal		7.33±0.82	0.44±2.14	41.83±1.17	58.5±1.05	121.17±1.94
Control	-	4.67±1.03	0.96±1.41	102.67±1.75	113.67±1.21	199.00±1.41
CAME-I	250	6.50±0.84	0.62±1.17	71.67±1.63	66.67±1.75	155.83±1.47
CAME-II	500	7.33±0.52	0.52±1.17	47.33±1.21	59.17±1.17	126.33±1.63
GBL	10	7.50±0.55	0.39±1.21	43.83±1.17	57.33±1.21	123.33±1.63

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Data represent means± S.E.M. *P<0.05, **P<0.01.

Table 5

Effect ofCelosia argenteaon kidney parameters in normal and diabetic rats (n=6).

Groups/ Treatments	DOSE (mg/kg)	Serum urea (mg/dL)	Serum creatinine (mg/dL)
Normal		31.00±0.89	0.60±0.42
Control	-	60.83±1.18	0.96±1.15
CAME-I	250	44.00±1.10	0.88±0.93
CAME-II	500	36.00±0.89	0.72±1.87
GBL	10	34.17±0.75	0.64±1.03

Data represent means ±S.E.M. *P<0.05, **P<0.01.