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Research Article

AN EVALUATION OF THE ANTIOXIDANT AND ANTI-DIABETIC EFFECT OF THE METHANOL EXTRACT OF *HYPOXISHEMEROCALLIDEACORM* (MEHHC) ON STZ INDUCED SD RATS'

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disease characterized by chronic hyperglycaemia generally associated with oxidative stress. The present study aims at evaluating the antioxidant and anti-diabetic potential of methanol extract of the corm of *Hypoxis hemerocallidea* (MEHHC)commonly known as the 'African potato'. The study was conducted in two different secessions and first part estimated the antioxidant status and the second part was assessing the anti-diabetic properties The *in –vivo* study was designed to check the anti- hyperglycemic effect of the extract on a weekly basis and the other parameters checked at the end of the experiments were the liver markers lipid profile and other glycemic indices. The results of the first session showed higher levels of antioxidant status and the *in-vivo* studies showed the anti-hyperglycemic effects of the extract. This was comparable to the effect of the anti-diabetic drugs used for the treatment of diabetes. The overall study indicates that MEHHC is effective in controlling the diabetes through its antioxidant system.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by both postprandial and fasting hyperglycemia with disturbances in carbohydrate, fat and protein metabolism. DM results from defects in insulin secretion, action or a combination of both which is increasing globally and now affects 7% of the world's adult population(G Daily, 2011]. It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million individuals worldwide. The World Health Organization (WHO) has predicted that the number of patients with diabetes worldwide will double by the year 2025, from the current number of approximately 150 million to 300 million (Shaw J.E *et al,* 2010). It is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels (hyperglycemia) (Philippe J and Raccah D, 2009). Being a major degenerative disease, diabetes is found in all parts of the world and it is becoming the third most lethal disease of mankind and the numbers are increasing rapidly every year. Diabetes mellitus has been associated with an increased risk of mortality and prevalence of cardiovascular disease. Atherosclerotic cardiovascular disease is the main source of morbidity and mortality in patients with diabetes. (WHO2014).

It has been estimated that up to one-third of patients with diabetes mellitus use some form of complementary and alternative medicine. Complementary and alternative medicine involves the use of herbs and other dietary supplements as alternatives to mainstream western medical treatment. A recent study has estimated that up to 30% of patients with diabetes mellitus use complementary and alternative medicine (American Diabetic Association, 1997). Since ancient times, plants and plant extracts were used to combat diabetes. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. The World Health Organization (WHO) has listed 21 000 plants, which are used for medicinal purposes around the world. Among them, 150 species are used commercially on a fairly large scale. One of such medicinal plant used in southern Africa is the African potato. *Hypoxis hemerocallidea* (also known as *H. rooperi*). This popular 'miracle' medicinal plant is widely distributed in the southern Africa sub-region. The plant is characterized by strap-like leaves, and bright yellow, star-shaped flowers (Van Wyk B.E *et al,* 2002). The tuberous rootstock (i.e. the corm) of the plant is commonly referred to as 'African potato', because of its potato-like shape. Hypoxis is a family of plants that are extensively used for medicinal purposes in the Southern African region. This family consists of several types of species which include *H.interjecta*, *H. multiceps*, *H. nyasica*, *H. obtuse*, *H. sobolifera*, and the *H. hemerocallidea*. The hypoxidaceae super family

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ismade up of 8 genera and 130 species. Taxonomically, *Hypoxis hemerocallidea* belongs to the hypoxidaceae (Star lily family). This plant was first described by Linnaeus in 1759. The name was derived from the Greek words *hypo* (below) and *oxy* (meaning sharp), with reference to the ovary which is pointed at the base. The plant has recently drawn the attention of researchers because of its beneficial medicinal effects. Geographically, the plant is mostly distributed in the southern hemisphere and is mostly abundant in Southern Africa (Van Wyk B.E *et al,* 2011). The tuberous part of the *H. hemerocallidea* is the one that is believed to possess bioactive compounds. In folk medicine, the African potato had been used for centuries to treat many ailments such as arthritis, diabetes mellitus, high blood pressure, and cancer. The therapeutic effects of the African potato were attributed to the presence of sterols, sterols, sterolins, norlignan, daucosterol, and rooperol. (G.J Boukes *et al,*2008)

Even though many of studies conducted in this area could not reveal the actual mode of action of this plant. So The major aim of this study was, therefore, to examine the possible hypoglycaemic effect of methanolic extract of *Hypoxis hemerocallidea* (MEHH) in normal and diabetic rats, with a view of providing a pharmacological rationale for this extract in the management of adult-onset,Type-2 diabetes mellitus. The present study will give a clear idea of the mode of action in the anti-hyperglycemic effect of this plant to some of the South African traditional healers that uses this plant for the management of Type-2 diabetes mellitus.

MATERIALS AND METHODS

Collection and identification of plants

The underground corms were collected locally from Botswana and the identification of the plants were done by Dr. M. P. Setshogo at the University of Botswana Herbarium (UCBA) .The voucher specimen were submitted in the herbarium and voucher No. were (G2016/, A01).

Preparation of the extract: Methanol extract

The corms were cut into small pieces, dried in the shade, coarsely powered and soaked in 70% methanol for three days at room temperature. The extract was filtered and made it solvent free by using a Buchi type rotary evaporator (65 ^{o}C) and dried completely in the vacuum. The yields were 7.8%**.** The extract obtained was used to carry out the experiments as MEHHC (Methanol extract of *Hypoxis hemerocallidea*corm)

Chemicals

All the chemicals used were analytical grade and bought from Sigma-Aldrich Chemical Company, (St. Louis, MO) USA. Reagents DPPH (2,2-diphenyl-l-picrylhydrazyl) reagent [molecular formula C18H12N5O6 molecular weight 394 g/mol] was purchased from Fluka Chemicals (Steinheim, Germany), Ascorbic acid and anhydrous sodium carbonate were all analytically pure and were purchased from Unilab (South Africa). Gallic acid (AR) was obtained from Sigma Chemicals (Steinheim, Germany). The solvents used for the extraction process were also of analytical grade.

Animals

Male albino rats of Wistar strain (*Rattusnorvegicus*) of body weight ranging 200-250 grams were housed in colony cages at ambient temperatures of 25 o C ± 2 o C and 50-55% relative humidity with 12 Hours light and dark cycle. They had water and food *ad libitum.* The experiment was conducted as per the internationally accepted principles for laboratory animal care unit of the University of Botswana.

Statistical analysis

All data were expressed as the mean ±S.E. mean of n=5. Analysis of variance was performed by one-way ANOVA and the significant difference between the means were determined by the Holm-Sidak method. The *p* value ≤0.05 was regarded as significant. In all these cases, Statistical Software Stata 13.1 was used to analyze the data.

Antioxidant Status

2, 2-Azobis-3-ethyl benzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity

ABTS radical scavenging activity was determined by the method described by Pellegrini *et al.*,

The ABTS radical cations are produced when ABTS (7 mM) reacts with potassium persulfate (2.45 mM) when incubated at room temperature in the dark for 16 hrs. The solution thus obtained was further diluted with phosphate buffer saline (PBS) to give an absorbance of 1.000. Different concentrations of the test sample in 50 µl were added to 950 µl of ABTS working a solution to give a final volume of 1 ml. The absorbance was recorded immediately at 734 nm. Gallic acid was used as reference standard. Inhibiting concentrations of extracts were tested at 2.5, 5, 10, 25, 50 and 100µg/ml. Reference standard (gallic acid) was tested at 1, 2, 4, 8 and 16 µg/ml. The percent inhibition was calculated from the following equation:
% inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance
Control] x100.

DPPH ASSAY Spectrophotometric Method

The free radical scavenging activity was measured using DPPH method modified by Yeboah and Majinda (2009). Solutions of 500 µM DPPH (i.e. 0.02 % or 0.2 mg/mL) in methanol (AR) was prepared. Also different concentrations of each of the plant extracts and standards were prepared (ascorbic acid and gallic acid) ranging from 0.001-0.05 mg/mL in methanol. Each extract or standard solution (2 mL) was added to an equal volume of the DPPH solution, making a total reaction volume of 4 mL. A control reaction mixture was prepared consisting of 2 mL methanol without extract and an equal volume of DPPH solution. The test tubes were tightly closed, vigorously shaken and placed in a dark cupboard for 30 minutes. The absorbance of each solution was measured at 517 nm, and methanol was used as the blank for baseline correction, after 2 h and finally after 24 h. The percentage inhibition of DPPH, I % was calculated using the following formula:

$$I\% = \frac{(\text{Absorbance control} - \text{Absorbance sample}) \times 100}{\text{Absorbance control}} \quad (7)$$

From the inhibition curves (I % versus sample concentration in µg/mL) the concentration of extract or standard required to inhibit DPPH radical activity by 50% (IC_{50}) was determined from non-linear regression equations that best fitted the curves. The experiment was carried out in triplicate and the IC_{50} values reported as the average of three trails in µg/ mL + the standard deviation.[138]

Thiobarbituric acid (TBA) assay

The method followed was described by Rezaeizadeh *et al.* (2011) Briefly, extracts (1 mg/mL, 2 mL) were added to aqueous trichloroacetic acid (20 %, 1 mL) and thiobarbituric acid (0.67%, 2 mL). After boiling for 10 min. the mixture was cooled and centrifuged at 3,000 rpm for 30 min. The absorbance of the supernatant was recorded at 532 nm. The antioxidant activity was calculated by the percentage of inhibition as follows: % Inhibition = 100-[(A1-A0) ×100]. Where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts. Measurements were done in triplicates.

Acute Oral toxicity test

Experimental rats of either sex will be subjected to acute toxicity test. Different doses of extracts (50 -2000 mg/kg body weight) will be administered orally to experimental rats. For the first two hours, the animals were observed for the gross behavioral changes and then for seven days close observation of the animals checking the mortality. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia and mortality will be observed. (Patel P, *et al,* 2012)

Antidiabetic Properties

Assessing the effects of plant extract on ORAL GLUCOSE TOLERANCE (OGTT) in normal rats

Oral glucose tolerance test will be performed with different doses of extract in different groups of rats against the glucose load of 2g kg^{-1}In this test, 30 normal rats were randomly categorized into 6 groups (*n*=5). After an over- night fast, Group 1 is the normal control and group 2 and 3 which consisted of the positive controls were given metformin (500 mg/kg body weight) and glibenclamide (600 mg/kg body weight respectively). Group 4-6 accordingly received single oral doses of 100, 200 and 300 (mg/Kg body weight) of MEHC respectively. Blood was collected from tail vein before (0 min) and at 1h, 2h, 3h, 5h and 7h post treatment for glucose measurement.

Acute Glucose Test in Normal Rats

In this test, 30 normal rats were randomly categorized into six groups (*n*=5). rats were given single dose only. After an overnight fast, rats were categorized into following groups:

Group 1: normal control: NC (normal diet with distilled water).
Group 2; positive control: PC 1 (treated with metformin500 mg/kg body weight/rat/day)
Group 3; positive control: PC 2 (treated with glibenclamide 600 mg/kg body weight./rat/day).
Group 4: Experimental rats EX-1 Experimental rats administered with extract (100mg/Kg body weight./rat/day).
Group 5: Experimental rats EX-2 Experimental rats administered with extract (200mg/Kg body weight./rat/day).
Group 6: Experimental rats EX-3 Experimental rats administered with extract (300mg/Kg body weight).

Blood was collected from tail vein before (0 min) and at 1 h, 2 h, 3 h, 5 h and 7 h post treatment for glucose measurement.

Diabetic induction

Prior to diabetes induction, an optimum STZ dose selection study was carried out to determine the appropriate dose that will produce the needed chronic hyperglycemia, but with moderate mortality. To 6 groups (*n*=4) of overnight fasted rats (200-250 g), varying doses of STZ (65, 60, 55, 50, 45 and 40 mg/kg) reconstituted in freshly prepared buffer (0.1 mol/L cold citrate buffer of pH 4.5) were administered intra-peritoneal. These rats were monitored for 12 d for mortality, and the blood glucose level was measured on the first and last days. Clinical features of diabetes including polyurea, polyphagia, polydipsia and glycosuria were also observed.

Diabetes was induced in r*a*ts by intraperitoneal injection of 40 mg/kg of streptozotocin (STZ, Sigma-Aldrich Chemical Co., USA) reconstituted in 0.1 mol/L cold citrate buffer (pH 4.5) after an overnight fast. After 72 h of STZ administration, blood glucose level was measured in blood collected from tail vein puncture using Accu-check Advantage II clinical glucose meter (Roche Diagnostics Co., USA). Rats with FBG≥15 mmol/L (270 mg/dL) were considered diabetic and included in the study.

The percentage change in blood glucose was calculated thus:
Percentage of clycaemic change=(G*x*- G*i*)/G*x*×100
Where G*x* is the glycaemia at time *x* and G*i* is the glycaemia at the initial time (*i*).

Acute/single dose glucose response test in STZ-induced diabetic rats

In this test, 30 normal rats were randomly categorized into five groups (*n*=5). After an overnight fast,

Group 1: normal control: NC (normal diet with distilled water.
Group 2; diabetic control: DC (diabetic induced).
Group 3; positive control: PC 1 (treated with metformin500 mg/kg body weight/rat/day)
Group 4; positive control: PC 2 (treated with glibenclamide 600 mg/kg body weight./rat/day).
Group 5: Experimental rats: EX-1 Experimental rats administered with MEHHC (100mg/Kg body weight).
Group 6: Experimental rats: EX-2 Experimental rats administered with MEHHC (200mg/Kg body weight).
Group 7: Experimental rats: EX-3 Experimental rats administered with MEHHC (300mg/Kg body weight).

Blood was collected from tail vein before (0 min) and at 1 h, 2 h, 3 h, 5 h and 7 h post treatment for glucose measurement.

In-Vivo Analysis

Experimental design

All the following groups except group 1 were treated after inducing diabetes with STZ

Group 1: NC-Normal Control (rats received normal diet and distilled water)
Group 2: DC Diabetic control (rats received 55 mg streptozotocin (STZ)/Kg body weight).
Group 3: Positive control- PC-1– (rats administered with metformin 500mg/Kg body weight.)
Group 4: Positive control- PC 2- (Diabetic rats administered with glibenclamide (600 mg/kg
b.w./rat/day) in aqueous medium orally for 30 days (Pari and Umamaheswari 2000)
Group 5: Positive control- PC 3- (rats administered with metformin 500mg and 2mgAmaryl /Kg body weight.)
Group 6: Experimental rats EX-1 Experimental rats administered with extract (200mg/Kg body weight).
Group 7: Experimental rats EX-2 Experimental rats administered with extract (300mg/Kg body weight).

Treatment was carried out for 30 days and at the end of the experimental period final fasting blood glucose levels and body weights were recorded. Rats were anaesthetized and blood samples were collected from the aorta. Then the blood was centrifuged at 3500 rpm for 15 minutes. Once centrifuged, the plasma was transferred into cryotubes, frozen in liquid nitrogen and stored at 80 degrees Celsius for biochemical estimations.

Biochemical analysis

TBARS was estimated by the method described by Niehaus and Samuelsson (1986).Glucose levels were determined by the use of the glucometer. Hb1Ac, total proteins, and total albumen were estimated by kits from Agape Switzerland and their instructions were strictly followed. Alanine aminotransferase (ALT), alanine as partatetransaminase (AST) alkaline phosphatase (ALP) were estimated by the kits bought from Sigma and the manufacture's guidelines were followed. The lipid profile was done with kits bought from Agape Switzerland and their instructions were strictly followed. This includes the triglyceride, LDL, HDL and total cholesterol.

Determination of the Relative Tissue Weights

Relative tissue weights were estimated by comparing the tissue weight to the total body weight (relative kidney/liver weight): Relative weight =tissue weight (g)/total body weight (g)× 100.

RESULTS

ABTS Assay

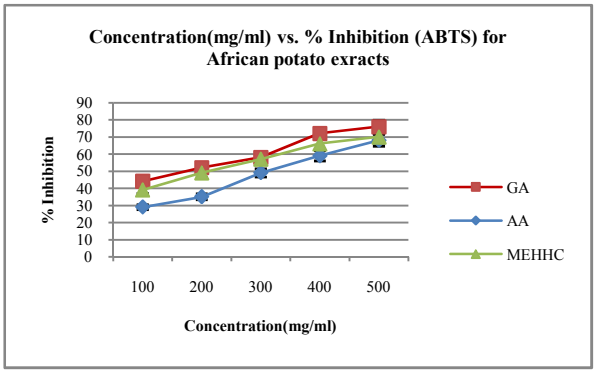

Fig 1 % inhibition of ABTS for MEHHC
GA-Gallic acid, AA Ascorbic acid, MEHHC Methanol extract of *Hypoxis hemerocallidea*

This method is used for the screening of antioxidant activity is reported as a decolourisation assay applicable to both lipophilic and hydrophilic antioxidants, The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity.

DPPH Assay

This method has been used extensively to predict the antioxidant activities due to its relatively short time required for analysis. Fig 1 shows the radical scavenging activity of MEHHC with DPPH and the results illustrates that the radical scavenging activity of the extract is increasing the concentration.

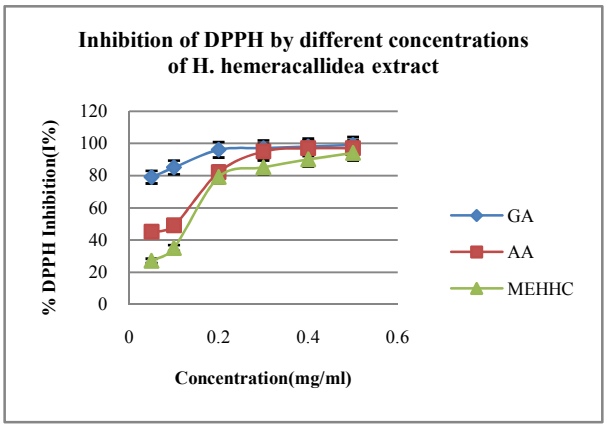

Fig 2 % inhibition of MEHHC in DPPH assay

GA-Gallic acid, AA Ascorbic acid, MEHHC Methanol extract of *Hypoxis hemerocallidea*

TBA Assay

Malondialdehyde assay is the most generally used test in the appreciation of the role of oxidative stress in disease. Malondialdehyde is one of the several products formed during the radical induced decomposition of polyunsaturated fatty acids. The methanol exhibits a good range of activity in % inhibition at a dose of 1 mg/ml. the activity of the methanol extract is more than that of VIT C but lower than that of BHT Butylate hydroxyanisole which is a synthetic antioxidant.

Table 1 % Inhibition of TBA assay

EXTRACT	% INHIBITION AT 30 MIN	% INHIBITION AT 1 HOUR
BHT	95.66±.87	96.22±.05
VIT C	23.45±.12	25.06±.43
MEHHC	70.56±.05	76.53±.09

Acute Toxicity

The extract administered up to high dose (4 000 mg/kg) produced no mortality. The animals did not manifest any sign of restlessness, respiratory distress, general irritation, coma or convulsion. Hence this extract was considered safe for rats.

OGTT in Normal Rats

Table: 2shows the blood glucose levels of control and experimental groups of rats after oral administration of glucose. The blood glucose level in the control rats rose to a peak value 60 min after a glucose load and decreased to near normal levels

after 120 min. Metformin and glibenclamide treated rats showed a significant decrease in blood glucose concentrations at 60 and 120 min compared to the experimental groups. The effect of the extracts is almost like the standard drugs for diabetes in dose dependent manner.

The blood glucose level in the control rats rose to a peak value 60 min after a glucose load and decreased to near normal levels after 120 min. In diabetic control rats, the increase in blood glucose was observed after 60 min and remained high forever.

Table 2 OGTT in Normal Rats

		Glucose level (mmol/L) over time(hours)					
Experiment	**Group**	**0hr**	**1hr**	**2hr**	**3hr**	**5hr**	**7hr**
CONTROL	**NC**	5.16±0.22	5.23±0.27	5.31±0.06	5.09±1.02	5.35±0.54	5.08±0.43
	PC	4.51 ±01	7.56±0.09*	6.36±0.04	5.76±0.09	5.32±0.08	5.22±0.05
	EX 1	4.94±0.06	6.78±0.12*	6.72±.0.23*	6.46±0.11	6.12±0.07	5.46±0.07
EXPERIMENTAL	**EX 2**	4.72±.0.06	6.63±.1.08	6.54±0.62	5.88±0.67	5.73±0.04	5.06±0.03
	EX 3	4.98±0.09	6.23±0.83	5.85±0.82	5.56±0.73	5.02±0.76	4.76±0.05

n= 6in each group,* shows significant difference and the results were given as mean ±SEM.P ≤0.05

NC-Normal control, PC-positive control, glucose only, EX- 1 Experimental treated with extract100mg/Kg body weight, EX- 2 Experimental treated with extract 200mg/Kg body weight, EX- 3 Experimental treated with extract 300mg/Kg body weight

Acute Glucose Test in Normal Rats

Both doses of methanol extract and glibenclamide significantly reduced fasting blood glucose level. Glibenclamide and metformin showed a significant reduction at the level of P<0.01. MEHHC showed a significant reduction at the level of P<0.05 and P<0.01 respectively. These results suggest that hypoglycemic activity of 300 mg/kg dose and glibenclamide has similar significance level.

MEHHC treated groups showed significant reduction in the glucose level stating at the 60 minutes or may be before and glibenclamide treated diabetic rats showed a significant decrease in blood glucose concentrations at 60 and120 min compared with diabetic rat

NC-Normal control, DC- Diabetic control, PC 1-positive control (treated with metformin500 mg/kg body weight/rat/day), positive control: PC 2 (treated with glibenclamide 600 mg/kg body weight./rat/day).

EX- 1 Experimental treated with extract 100mg/Kg body weight, EX- 2 Experimental treated with extract 200mg/Kg body weight, EX- 3 Experimental treated with extract 300mg/Kg body weight

Table 3 levels of glucose in acute glucose test in normal rats

		Glucose level (mmol/L) over time(hours)					
Experiment	**Group**	**0hr**	**1hr**	**2hr**	**3hr**	**5hr**	**7hr**
CONTROL	**NC**	5.28±0.11	5.58 ±0.15	5.48 ±0.14	5.72±0.15	5.78 ±0.15	5.52 ±0.08
	PC 1	4.44±0.11	6.92±0.15*	6.26 ±0.19	5.58 0.15	5.31 ±0.15	5.02 ±0.08
	PC 2	4.48±0.17	6.82±0.65*	6.25±0.14	5.98±0.18	5. 81±0.15	5.32±0.28
	EX 1	5.01±0.11	7.12±0.15*	6.9 ±0.14*	6.56 0.15	6.14 ±0.15	5.48±0.08
EXPERIMENTAL	**EX 2**	4.7 ±0.11	6.42±0.15	6.08 ±0.14	5.7 ±0.15	5.22 ±0.15	5.06 ±0.08
	EX 3	5.03±0.87	6.02±0.13	5.98 ±0.18	5.54±0.25	5.02 ±0.17	4.98±0.06

n= 5 in each group,* shows significant difference and the results were given as mean ±SEM.P ≤0.05

NC-Normal control, PC 1-positive control (treated with metformin500 mg/kg body weight/rat/day), positive control: PC 2 (treated with glibenclamide 600 mg/kg body weight./rat/day).

EX- 1 Experimental treated with extract 100mg/Kg body weight, EX- 2 Experimental treated with extract 200mg/Kg body weight, EX- 3 Experimental treated with extract 300mg/Kg body weight

Acute Glucose Test in Diabetic Rats

Table:4 shows the blood glucose levels of control and experimental groups of rats after oral administration of glucose.

In -Vivo study

Blood glucose level –a weekly response

The table presents the glycemic effects MEHHC in streptozotocin- induced diabetic rats. There was a continuous increase in the fasting blood glucose level of diabetic control rats from the first week of treatment till the fourth week. While, upon oral administration MEHHC, there was a significant reduction (p<0.05) in the fasting blood glucose level, of rats administered 200 and 300 mg/kg body weight of the extract in a dose-dependent manner, decline in the fasting blood glucose concentration began on week one and continued till the fourth

Table 4 levels of glucose in acute glucose test in diabetic rats

		Glucose level (mmol/L) over time(hours)					
Experiment	**Group**	**0hr**	**1hr**	**2hr**	**3hr**	**5hr**	**7hr**
	NC	5.28±0.11	6.58 0.15	6.48 ±0.14	6.22±0.15	5.78 ±0.15	5.52 ±0.08
CONTROL	**DC**	18.96±0.29*	19.00±0.34*	18.52±0.42*	18.72±0.34*	17.92±0.25*	18.16±0.26*
	PC 1	18.82±0.29	17.80±0.34	16.16±0.4	15.02±0.34	13.76±0.25	10.64±0.26
	PC 2	18.46±0.29	16.30±0.34	13.50±0.4	11.92±0.34	10.84±0.25	9.12±0.26
	EX 1	18.78±0.29	15.68±0.14	14.47±0.44*	13.18±0.04*	11.96±0.95*	10.40±0.06*
EXPERIMENTAL	**EX 2**	18.40±0.29	14.62±0.34*	13.44±0.04*	11.14±0.34*	9.36±0.25*	8.42±0.26*
	EX 3	17.98±0.29	14.46±0.34*	10.56±0.4*	9.22±0.34*	8.06±0.25*	6.48±0.25*

n= 6in each group,* shows significant difference when compared with DC and the results were given as mean ±SEM.P ≤0.05

week. Whereas, the continuous reduction was recorded for fasting blood glucose concentration in 300 mg/kg body weight which was similar to metformin glibenclamide combination.

decrease was observed in HbA1c level in diabetic rats after treatment with MEHHC when compared with DC groups at the end of experimental period.

Table 5 Blood glucose level a weekly response

Experiment	Group	Glucose level (mmol/L)			
		Week 1	Week 2	Week 3	Week 4
CONTROL	NC	5.16± 0.27	5.24±0.32	5.08±0.28	4.96±0.24
	DC	17.54± 0.27*	17.72±0.32*	17.54±0.28*	17.52±0.08*
	PC 1	14.36± 0.27*	12.36±0.32*	11.46±0.28*	9.38±0.93
	PC 2	13.92± 0.27*	11.66±0.32	10.98±0.28*	7.32±0.67
	PC3	11.66± 0.27*	8.60±0.32	7.10±0.28	6.46±0.87
EXPERIMENTAL	EX 1	15.66± 0.27*	14.26±0.12*	13.20±0.88	11.08±0.97
	EX 2	13.66± 0.27*	12.20±0.32*	10.40±0.28	6.88±1.32
	EX 3	12.16± 0.27*	9.64±0.32	7.86±0.28	5.62±1.09

n= 6in each group,* shows significant difference and the results were given as mean ±SEM.P ≤0.05

NC Normal control, DC Diabetic control, PC Positive control, EX Experimental groups

Relative body and organ weight

Table 6. Shows the body weight of control and experimental groups of rats. A significant decrease in body weight was observed in streptozotocin (STZ) induced diabetic rats (DC Group) when compared to the control rats showed a progressive increase in body weight which was near normal at the end of experimental period.

Effect of MEHHC on total protein

An overall reduction in serum total protein in diabetic animals and subsequent reduction in the albumin was observed in the present study.

Table 6 Effect of **MEHHC** on total body weight and relative weights of kidney and liver tissues.

Experiment	Group	Total body weight	Liver weight	Kidney weight	elative liver weight	Relative Kidney weight
CONTROL GROUPS	N C	302.54± 43	10.78±.93	1.98 ±0.38	3.56±0.31	0.65±0.44
	DC	214.34±.41	10.08±.87	2.54±0.57	4.71±0.28	0.83±0.95
	PC 1	278.67±.84	10.04±.76	2.16±0.83	3.60±2.82	0.77±0.67
	PC2	282.66±.62	10.43±.72	2.13±0.67	3.68±0.99	0.75±0.53
	PC3	301.76±.42	10.66±.87	1.78±0..43	3.53±0.65	0.59±1.36
EXPERIMENTAL	EX1	279.86±.93	10.03±.95	2.34±0..36	3.58±0.83	0.92±0.67
	EX2	279.31±52	10.23±.82	2.04±0.85	3.66±0.66	0.73±0.11
GROUPS	EX3	303.43±.95	10.81±.76	1.96±0.93	3.56±2.65	0.64±0.56

n= 6in each group, and the results were given as mean ±SEM.P ≤0.05

NC Normal control, DC Diabetic control, PC Positive control, EX Experimental groups

Effect of MEHHC on TBARS

Lipid peroxidation was measured as malondialdehyde (MDA) level in the serum. The results indicate the preventive effects of MEHHC on lipid peroxidation. The levels of TBARS were significantly increased in diabetic animals as compared to control animals. The levels of TBARS were significantly reduced in diabetic animals treated with MEHHC.

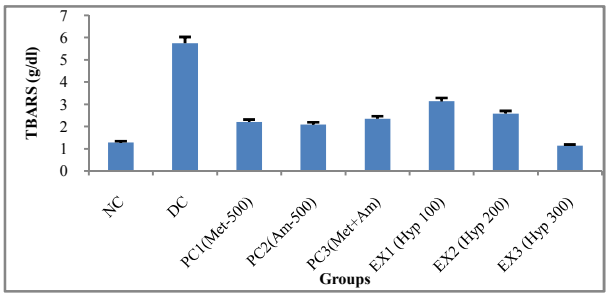

Fig 3 Effect of MEHHC on TBARS

n= 6in each group,* shows significant difference and the results were given as mean ±SEM.P ≤0.05

NC Normal control, DC Diabetic control, PC Positive control, EX Experimental groups

Effect of MEHHC on HB1Ac

An increase in the levels of serum HbA1c in STZ- treated rats when compared with NC and PC groups. A significant

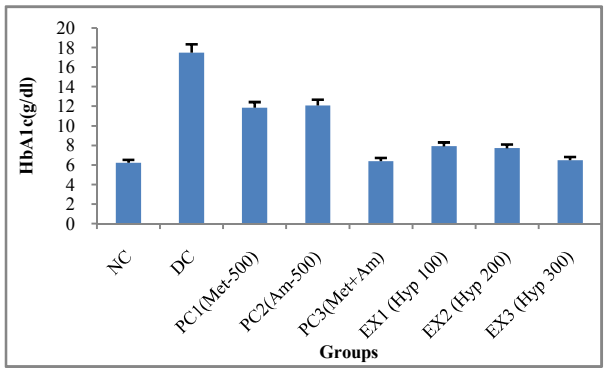

Fig 4 Effect of MEHHC on HB1Ac

n= 6in each group,* shows significant difference and the results were given as mean ±SEM.P ≤0.05

NC Normal control, DC Diabetic control, PC Positive control, EX Experimental groups

Effect of MEHHC on albumen

The levels were significantly lowered in DC group c compared to the NC group.

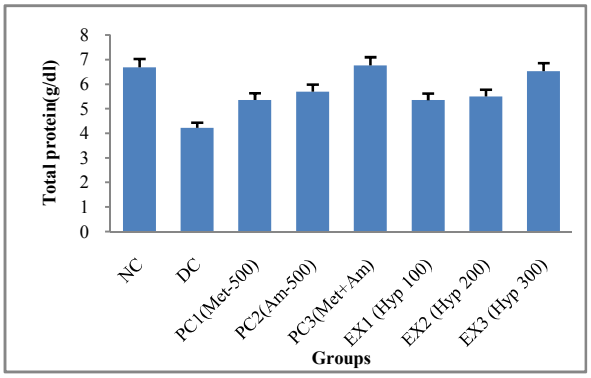

Fig 5 Effect of MEHHC on total protein

n= 6in each group,* shows significant difference and the results were given as mean ±SEM.P ≤0.05

NC Normal control, DC Diabetic control, PC Positive control, EX Experimental groups

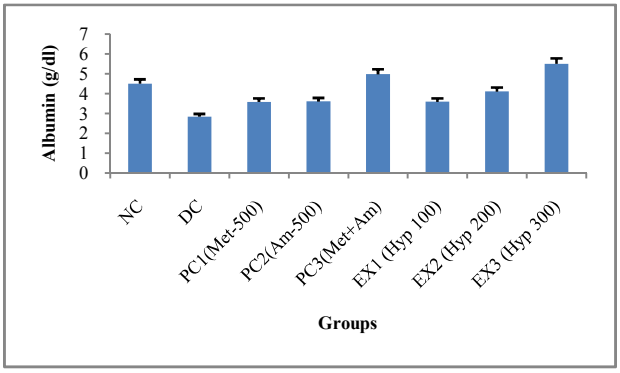

Fig 6 Effect of MEHHC on albumen

= 6in each group,* shows significant difference and the results were given as mean ±SEM.P ≤0.05

NC Normal control, DC Diabetic control, PC Positive control, EX Experimental groups

Levels of liver markers in plasma

The results reflected in (Table7) indicate the liver function indices of alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatases (ALP), total bilirubin(TP) concentrations in the serum after 30 days of oral administration of the extract MEHHC. There was a significant (P<0.05) increase in the activities of all these liver marker enzymes (ALT, AST, and ALP) in the diabetic-induced control group (DC) when compared with the NC and EX groups.

Lipid profile

Serum total cholesterol (TC) of the DC group was not significantly different (p >0.05) from that of the NC group

All the following groups except group 1 were treated after inducing diabetes with STZ

Group 1: NC-Normal Control (rats received normal diet and distilled water)

Group 2: DC Diabetic control (rats received 55 mg streptozotocin (STZ)/Kg body weight).

Group 3: Positive control- PC-1– (rats administered with metformin 500mg/Kg body weight.)

Group 4: Positive control- PC 2- (Diabetic rats administered with glibenclamide (600 mg/kg b.w./rat/day) in aqueous medium orally for 30 days (Pari and Umamaheswari 2000)

Group 5: Positive control- PC 3- (rats administered with metformin 500mg and 2mgAmaryl /Kg body weight.)

Group 6: Experimental rats EX-1 Experimental rats administered with extract (200mg/Kg body weight).

Group 7: Experimental rats EX-2 Experimental rats administered with extract (300mg/Kg body weight).

DISCUSSION

Diabetes is increasing at an alarming rate worldwide which is mainly due to the lifestyle and calorie rich food. Diabetes is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbance of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Many oral antihyperglycemic agents, such as sulfonylurea and biguanides, are available along with insulin for the treatment of diabetes, but these agents have significant side effects, and some are ineffective in chronic diabetes patients. Thus, there is an increasing need of new natural anti-hyperglycemic products especially nutraceuticals with fewer side effects, safe, and high antihyperglycemic potential. (Patel P, *et al,* 2012). Generation of the ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of solutions of pure substances.

Table 7 Levels of liver markers in plasma

Experiment	Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	(TB) (mg/dl)
Control Groups	N C	40.63±**1.02**	41.64±0.89	63.47±1.33	6.34±1.07
	DC	91.22±0.89*	91.22±0.08*	164.02±1.09*	12.02±0.06*
	PC 1	60.13±1.02	55.19±0.09	95.84±1.45*	7.84±1.63
	PC2	57.93±1.02	54.63±0.45	92.05±1.39*	6.64±0.93
	PC3	39.20±1.02	40.60±0.78	73.89±1.73	6.34±1.04
Experimental Groups	EX1	59.52±1.02	63.53±0.69	87.16±0.33*	7.64±1.13
	EX2	45.36±0.09	43.33±0.84	67.63±0.39	6.44±1.83
	EX3	40.36±1.05	38.03±0.49	59.03±0.34	6.42±1.58

n= 5in each group,* shows significant difference and the results were given as mean ±SEM.P ≤0.05

NC Normal control, DC Diabetic control, PC Positive control, EX Experimental groups

The EX groups did not show any significant difference in the liver marker enzymes when compared with the NC and the PC groups

The acute toxicity testing appeared normal and did not show any visible signs of toxicity and no mortality was observed during the test period. These studies revealed the non-toxic nature of MEHHC on normal rats.

Table 8 Lipid profile, total cholesterol, total LDL, total HDL and triglycerides

Experiment	Group	Total Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Total Triglycerides (mg/dL)
CONTROL GROUPS	N C	128.8± 2.14	38.43±1.33	65.21±1.02	132.02±0.89
	DC	644.27±1.04*	23.77±1.78*	112.38±1.22*	512.99±1.02*
	PC 1	321.49±0.94*	35.27±1.65	78.74±1.08	308.86±0.86*
	PC2	313.43±0.17*	34.91±0.93	78.08±0.22	312.13±1.52*
	PC3	137.78±1.94	39.45±1.42	87.26±0.29	277.35±1.92
EXPERIMENTAL	EX1	231.43±0.56	40.71±0.07	77.40±0.96	313.91±0.82*
	EX2	140.63±0.19	46.54±0.05	77.08±1.02	232.99±0.32
GROUPS	EX3	130.13±1.14	34.14±1.05	57.58±0.28	128.09±1.80

n= 6in each group,* shows significant difference and the results were given as mean ±SEM.P ≤0.05

NC Normal control, DC Diabetic control, PC Positive control, EX Experimental groups

Diabetes mellitus is associated with rapid weight loss as a consequence of uncontrolled catabolism of structural proteins as a compensatory response against abnormal carbohydrate metabolism. Muscle atrophy in diabetic subjects is due to a combination of decreased protein synthesis, increased gluconeogenesis, and increased protein degradation (Ono S.*et al,* 2015)

Table 1 shows the effect of the extract on the relative weights of the animals and specific organs. There was a significant loss of weight was noticed in STZ- induced diabetic rats when compared to NC groups. But there was no significant reduction in the relative weights of the kidney and the liver (Rajkumar I and Govindarajulu P, 1991). The decrease in body weight in diabetic rats clearly shows a loss or degradation of structural proteins due to diabetes as the structural proteins are known to contribute to body weight. Ravi *et al*. (2004) reported that the characteristic loss of body weight associated with STZ-induced diabetes is due to excessive breakdown of tissue proteins and an increased muscle wasting in diabetes. Additionally, several studies showed that the levels of serum total protein were declined in diabetic animals.[20] Plant extract administration improved the body weight in diabetic rats. This prevention of loss in body weight by MEHH C extract may be due to increase glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein or by glycemic control. Thus the plant extract has the potentials in improving the protein production by stimulating the insulin secretion.

Tables 2 indicates the OGTT results clearly led to definitively lower peak blood glucose values in 60 min after a glucose load and after 120 min. MEHHC might enhance glucose utilization because they significantly decreased the blood glucose level in glucose-loaded rats. It is very important to note that extracts showed a more pronounced action in the glucose tolerance test. (Gupta R and Gupta R.S, 2009) Glibenclamide is often used as a standard antidiabetic drug in STZ-induced moderate diabetes to be compared with a variety of hypoglycemic compounds and its effectiveness is known and standardized

Table 3 and 4 showed the acute glucose test in normal and diabetic rats. The capacity of MEHHC in decreasing the elevated blood sugar level to normal glycemic level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. A significant decrease in the level of glucose was observed in the EX groups. When compared with DC group but the extract treated groups showed this effect in a dose-dependent manner. The lower dose did not show much difference with the DC groups.

The higher level such as EX2 was in par with PC1 and PC2 which are the known drugs for diabetes. It may be due to restoration of delayed insulin response or due to an inhibition of intestinal absorption of glucose. The group EX3 showed the maximum effect and was comparable to NC groups. The previous experiments were showing the effects of the extract in a dose-dependent manner which were in par with metformin and Glibenclamide. It was noted that the higher doses were more effective than these drugs used. So it was decided to use a combination group with both the drugs together (Gupta R and Gupta R.S 2009). That was the PC3 which showed the effect of both the drugs and all the results in this set of experiments the EX3 was similar to PC3. There were no significant difference between PC3, EX3 and NC groups. The *In-vivo* experiments conducted estimated the weekly glucose level and this assessment showed the effect of this extract in a dose-dependent manner as well. The glucose levels were declining in all the groups except the DC groups which was untreated. *HBA1c* During diabetes the excess of glucose present in blood reacts with hemoglobin to form glycosylated hemoglobin The rate of glycosylation is proportional to the concentration of blood glucose Hence estimation of glycosylation of hemoglobin is a well-accepted parameter useful in the management and prognosis of the disease (V Radoi.*et al,* 2012)

The effect of administration of M EHHC extracts and the drugs used tried to bring the parameters significantly towards normal values. Hyperglycemia is the main cause for elevated free radical levels, followed by production of ROS, which can lead to increased lipid peroxidation and altered antioxidant defense and further impair glucose metabolism in a biological system. An imbalance between oxidation and antioxidant status has been shown to play an important role in mediating insulin resistance. Lipid peroxidation measurement is a more practical and safer method to evaluate the factors causing. Tissue MDA content, the final product of lipid breakdown caused by oxidative stress, is an important indicator of free radical-induced lipid peroxidation. (M.S. Balasubhashini. *et al,* 2004) Lipid peroxidation was indicated by TBARS, which were elevated to four times more when compared to the normal control. An increase in lipid peroxidation as measured by formation of TBARS or diene conjugates induces mitochondrial dysfunction in rats subjected to oxidative stress induced by diabetes[22]. The concentrations of total protein and albumin may indicate the state of the liver and type of damage caused by diabetes. Increase serum albumin concentrations in the diabetic control rats and the reduction following oral administration of MEHHC indicated the

amelioration of the adverse effects caused by diabetes. (AEidi M. Eidi and E.Esmaeili, 2006)

The liver markers such as ALT, AST and ALP are the specific markers to assess hepatocellular damage leading to liver cell necrosis. Thus lowering of these enzymes content in serum is a definite indication of hepatoprotective action of the extracts and drugs used. The extracts may induce accelerated regeneration of liver cells by reducing the leakage of AST into blood thereby lowering its value to normal levels. ALT is more specific to the liver and a better parameter for detecting liver damage. Bilirubin is formed by the breakdown of hemoglobin in the liver, spleen and bone marrow. (S.A. Gometi *et al,* 2014) The significant increase in the total bilirubin, and albumin levels in the diabetic control rats and reduction following oral administration of MEHHC are indicative of amelioration of the adverse effects caused by diabetes.

The changes in the lipid profile of normal and diabetic rats are illustrated in Table: 6. Untreated diabetic rats were characterized by a significant elevation in the levels of total cholesterol, LDL HDL and triacylglycerol's, compared with the normal animals. Administration of diabetic drugs, as well as the extract, caused a significant decrease in the serum level of LDL cholesterol when compared with diabetic rats. Significantly ($p<0.05$) decreased levels of serum HDL cholesterol were observed in diabetic rats when compared with the NC group. (B.Remesh and D Saralakumari, 2012, R.O Arise.*et al,* 2014).

Hyperglycemia is the main cause for elevated free radical levels, followed by production of ROS, which can lead to increased lipid peroxidation and altered antioxidant defense and further impair glucose metabolism in a biological system. The overall data in this study reveals the fact that *Hypoxis hemerocallideais*a plant with many antioxidant bioactive compounds which can effectively control the adverse effects of diabetes (Singh Y.1999). According to our study, the anti-diabetic activity is enhanced through the antioxidant system. The hyperglycemic state in diabetes has been attributed as the major factor that triggers the development of both acute and long-term changes in the cellular metabolism of different molecules. Altered metabolism of macromolecules ultimately triggers the excessive formation of free radicals via different pathways as a result speeding up the development of diabetic complications. It has been suggested that the ability of the 'African potato' extracts to suppress inflammation could be mediated via its antioxidant activity which, in turn, inhibits COX enzymes. (VDP Nair, *et al,* 2007) One of the most important chemical constituents of the herb which has been confirmed to be abundantly present in extracts of 'African potato' is a norlignan diglucoside, hypo side (M. Mohammed and J.O.A Ojewole2003). In the human gut, hypoconid is converted to roop Erol, a biologically active compound, by beta-glucosidase enzyme. Recent laboratory investigations have shown that roop Erol has a strong antioxidant activity, a strong affinity for phospholipid membranes, and that it inhibits free radical-induced membrane lipo-oxidation (G.J Boukes.*et al,* 2008). Most of the therapeutic properties of 'African potato' extracts observed clinically in man and in laboratory animals to date, have been attributed to roop Erol. So the results obtained in the present study also support that the norlignan diglucoside, hypoxoside present in MEHHC is a strong hyperglycemic agent working through the antioxidant system. (Oluwafemi. *et al,* 2016). 'African potato' extracts interestingly showed that only stigmasterol and rooperol had inhibitory effects on CYP3A4, CYP3A5and CYP19-mediated drug metabolism. Therefore, be at risk of developing adverse events which may lead to treatment failure, viral resistance and/or drug toxicity. (Mills E.*et al,* 2005, Willams J.A, 2002)

CONCLUSION

Based on the findings of this study the methanol extract of *Hypoxis hemerocallidea corm* showed

Anti -hyperglycemic effect in a dose-dependent manner. This activity is mainly through its antioxidant system. The data of our studies suggest that MEHHC is more beneficial in diabetes and its associated complications, holding hope of the new generation anti-hyperglycemic drug.

It was reported that higher doses interfere with other drugs and hence ultimately affects the renal system. Further studies need to be carried out to isolate and investigate the individual active principles. Some medicinal plants are known to possess both hyper- and hypoglycemic constituents. Therefore, isolation of the individual compounds would optimize the therapeutic effects of the plant.

Competing Interests

The authors declare that they have no competing interests

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