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Hypoglycaemic and Hypolipidaemic Effect of Extract of Lantana camara Linn. Leaf on Alloxan Diabetic Rats

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Abstract

Diabetes mellitus is a metabolic disorder with high prevalence worldwide and is a major medical concern. This study investigated the effect of extract and fractions of *Lantana camara* Linn. leaf on alloxan diabetes in rats. Phytochemical screening was carried out using standard qualitative procedures. The antidiabetic activity was evaluated using adult male albino rats induced with diabetes using 150mg/kg alloxan monohydrate. Daily oral administration of the extract and its fraction was via oral route for 28days. Estimation of glucose and lipid profile of rats was done using specific laboratory kits. Weekly monitoring of fasting glucose level was done using microprocessor digital blood glucose meter and accompanying strips. Column and planar chromatography was adopted for isolation of bioactive compounds in the fractions. Detection spray tests were employed for identification of isolated compounds. Carbohydrates, cardiac glycosides, flavonoids, polyphenols, sterols, saponins, tannins and triterpenoids were present in the extract and fractions. The seventy percent ethanol extract, aqueous and n-butanol fractions of *L. camara* Linn .leaf exhibited significant ($p<0.05$) hypoglycaemic activity. Hypolipidaemic effect was also observed. Triterpenes were isolated from the aqueous and n-butanol fractions. The study provided evidence of antidiabetic action of extract and fractions of *L. camara* Linn. leaf . Triterpenes are possibly responsible for the hypoglycaemic activity. Triterpenes and sterols come with great hope for discovery of new drugs for treatment of diseases such as diabetes.

Keywords: hypoglycaemia, hypolipidaemia, triterpenes.

1. Introduction

Diabetes mellitus is a metabolic disorder found in all nations of the world. It is one of the most prevalent epidemics of the 21st century, affecting citizens of both developed and developing countries (Mann& Hermansen 2004).World Health Organization (WHO) and African Regional Data estimates of 2007 showed that about 7million people had diabetes as at the year 2000 and estimated that 18 million are expected to come down with the disease in 2030.This rapidly increasing prevalence is a significant cause of concern. Despite the great strides that have been made in understanding and management of the disease, Diabetes mellitus and related complications continue to be a major medical problem. Worldwide, there is an increasing demand of natural products with antidiabetic activity and less side effects for treatment of the disease. Herbal medicines are a good option because of their comparable therapeutic effects. Substances derived from plants remain the basis for a large proportion of the commercial medications used today for the treatment of diseases.

Lantana camara Linn. (Family: Verbenaceae) has many common names including big sage, wild sage, ewon adele (Yoruba) anya nnunu (igbo) and kashin kuda (hausa). A native of the American tropics and sub-tropics, it has become naturalized in suitable habitats in tropical and warm regions worldwide. *L. camara* has several uses, different parts of the plant are used for medicinal and non-medicinal purposes. It has been listed among the useful plants of west tropical Africa by Burkill,(1985).Traditionally, the leaf of *L. camara* is used to treat eczema, chicken pox, rashes, boils, cold, malaria, fever headaches, sore throat, toothaches (Asprey 1953;Dalziel 1937;Elisabethsky&Castillios 1990).The plant has been reported as having pharmacological activities such as antibacterial activity, antioxidant activity, anti-inflammatory antipyretic activity, hepato-protective activity, anticancer activity, antitumor activity (Forestierti *et al*. 1996 Ghosh *et al.* 2010; Kaur *et al*. 2010).

The present study was undertaken with the aim of evaluating effect of extract of *Lantana camara* Linn. leaf on alloxan diabetes.

2. Experimental

2.1 Drugs and chemicals

Alloxan monohydrate, product of Sigma Aldrich, USA. Glibenclamide, product of Hovid BHD, Malaysia. All other chemicals used in this study were of analytical grade, product of Sigma Aldrich and Guangdong Guanghua Sci-Tech Co.Ltd,China.

2.2 Plant material

The leaf samples of the plant *lantana camara* Linn. were collected from new Afaka area of Igabi Local Government Area of Kaduna State. A sample of the plant was identified by a botanist/taxonomist in the herbarium of Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria, Kaduna State. (Voucher No.5). Fresh leaf samples of the plant *Lantana camara* Linn. were collected during the months of October-November. The collected leaf samples were air dried at room temperature under shade for about 4 weeks (28days).

2.3 Preparation of plant extract

The powdery form of the leaf was extracted with 70% percent ethanol.The scheme adopted for fractionation is a three-step multiple solvent partitioning scheme described by Gareth,(2007).100g of the extract (aqueous-ethanol) was solubilized in 1litre of distilled water. The aqueous solution was then successively partitioned using a series of solvents with increasing polarity; petroleum ether(60-80 ^{0}C), chloroform, ethyl acetate and n-butanol.

2.4 Animals

All rats used in this study were purchased from the animal house unit of Department of Pharmacology, Ahmadu Bello University, Zaria, Kaduna State and National Research Institute for Chemical Technology (NARICT), Basawa, Zaria, Kaduna State. The rats were adult male albino rats (wistar strain). The age of the rats varied between 3 and 4 months, and they weighed between 200g and 295g.

2.5 Antidiabetic activity

Diabetes was induced by a single intaperitoneal injection of freshly dissolved alloxan monohydrates (150mg/kg) to the rats fasted overnight prior to the experiment, allowing access to water only. Diabetes state was confirmed by measuring fasting blood glucose concentration(s) 72 hours after induction. Collection of blood samples from the rats was done using tail tipping method during the course of the experiment. A microprocessor digital blood glucose meter and accompanying strips was used for monitoring the blood glucose level(s) weekly. Rats having blood glucose level(s) above 11.1mmol/L (200mg/dl) were selected for the study. The extract (aqueous ethanol) and fractions were administered orally at a dose of 800mg/kg body weight to treated groups; II, III, IV, VI, VII & VIII. Rats in group IX (Positive control group) received 10mg/kg b.w glibenclamide while rats in Group I and V (negative control groups) received water *ad libitum*. Each group had 5rats. The treatment was administered daily for 28 days. All rats used for the experiment were sacrificed 24 hours after the last dose of treatment. Blood samples were collected into sample bottles devoid of anticoagulant. The blood samples were then centrifuged at 2,500rpm for 5minutes to obtain the sera.

2.6 Estimation of biochemical parameters

The determination of serum glucose, cholesterol, triglyceride, and high density lipoprotein cholesterol, were carried out using specific kits for each assay, product of Reckon Diagnostics P. Ltd, India. Low density lipoprotein cholesterol was calculated using the formular of Friedewald as specified by the Reckon Diagnostics P. Ltd kit used for High density lipoprotein cholesterol detemination.Weekly monitoring of fasting glucose level was done using microprocessor digital blood glucose meter and accompanying strips, Accu-chek active, product of Roche, China.

2.7 Isolation and identification procedure

Four grams of aqueous and n-butanol fractions of aqueous ethanol extract of *L.camara* leaf was placed in a 30cm length column with 2.5cm diameter. The column was packed with silica gel(60-120 mesh)**.**Gradient elution with 100% ethyl acetate, ethyl acetate: methanol(90:10)was used for separation of the compounds. The progress of the chromatographic separation was monitored by thin layer chromatography. The collected fraction(s) were analyzed using commercially prepared TLC Silica gel 60 F_{254} aluminium sheets, product of Merck, Germany. Similar fractions were pooled together. Preparative TLC was further used to purify the compounds.The isolated

compounds were dissolved in methanol and spotted on TLC plates. Butanol: acetic acid:water(8:1:1) was used for development. The plates were air-dried and sprayed with various detection sprays.Heating of the sprayed plates were done at 100^0C in the oven.

2.8 Statistical analysis

Statistical analysis were performed with the aid of Instat Statistical Package, Graph pad Software, Inc. USA. Data were expressed as mean ± standard deviation. The significance of difference(s) between the means of the treated and control groups were established by student's *t* test. $P < 0.05$ were considered to be significant.

3. Result and discussions

Chemical evaluation of the different phytochemicals of the extract and fractions of *L. camara* Linn. leaf revealed the presence of secondary metabolites which possess a wide range of activities, which can help in amelioration and protection against diseases. The presence of some of these pharmacologically active agents in the extract of *L. camara* is responsible for its medicinal properties. Cardiac glycosides, carbohydrates, flavonoids, saponins, sterols, tannins, triterpenoids and polyphenols were present in the extract and its polar fractions as shown in table 1. This preliminary phytochemical investigation showed the highly polar fractions to be richer in phytochemicals than the non-polar, hence their choice for antidiabetic activity investigation.

Alloxan is widely used to induce diabetes in experimental animals, causing the selective destruction of insulin-secretings β cells of the islets of Langerhans. The destruction of the insulin-secreting β cells is brought about by a redox cycle with the formation of superoxide radicals established by alloxan and the production of its reduced form; dialuric acid. Super oxide radicals undergo dismutation to hydrogen peroxide, which produces the reactive hydroxyl radicals by the Fenton reaction. The action of the reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration which leads to rapid destruction of β cells (Szkudelski 2001). This is accompanied by typical hypoinsulinemia and hyperglycaemia (Lenzen & Panten 1988)

Preliminary investigation of crude aqueous and ethanol extract to diabetic rats had a lowering effect on the fasting blood glucose level for all the doses(200,500,800,1000&1500) administered. The highest percentage reduction of 67.2% was recorded with 800mg/kg per body weight, hence the choice of this dose. Oral administration of 70% aqueous ethanol extract, its n-butanol and aqueous fractions reduced significantly($p<0.05$) the serum glucose levels in diabetic and normal rats (Table 2). Figure 1 shows the weekly glucose monitoring of the effect of oral administration of extract and fractions of *L.camara* Linn. leaf on diabetic state of rats. Comparing the changes in glucose level on weekly bases(0-7,7-14,14-21,21-28) showed significant reductions ($p< 0.05$).The extract and its fractions; n-sbutanol and aqueous reduced hyperglycaemia in the diabetic rats. A sharp drop was recorded on the 7^{th} day of treatment. From the 14^{th} to the 28^{th} day, the pattern of action of the aqueous fraction and the standard drug; glibenclamide was similar. The n- butanol fraction and the aqueous ethanol extract had similar pattern of action.

Diabetes is associated with a high risk to develop hyperlipidaemia. Hypercholesterolamia and high trigyceride levels was observed in rats used for this study due to their diabetic state. The obtained results suggest that the extract and fractions of *L. camara* Linn. leaf decreased the serum total cholesterol and triglyceride level in diabetic treated rats. However, not all reductions were significant. Plant sterols (phytosterols) have hydroxyl group at position C3 or double bond and the presence of an aliphatic chain at position C17. They are tricyclic compounds with varying degrees of saturation of the primary chain with difference in number and type of side substituents. Sterols are present in both free and bound-form of glycosides and esters. Sterols have many activities, including cholesterol- lowering effect, anti-cancer, anti-ulcer and allergic reactions inhibition(Berger *et al.*2004; Devaraj & Jialal 2006).Extracts and fractions of L. camara Linn. leaf tested positive for sterols. It can be speculated therefore that the extract of *L.camara* Linn. leaf have therapeutic potential against some of the lipid-mediated complications associated with diabetes mellitus.

Triterpenes are a large group of naturally occurring substances found in plants. They have cyclic structures composed of a carbon skeleton based on six isoprene units. They can be tetracyclic or pentacyclic. These compounds usually have one double bond and a secondary alcohol group. Their difference is in their distribution, number and type of oxygen functional groups. Cyclic triterpenes are non-volatile. lipophilic, and soluble in organic solvents.They react with acetic anhydride and concentrated sulphuric acid, exhibiting colour change,Lieberman Burchard reaction(Robinson 1991). The bioactive compounds isolated from the aqueous and n-butanol fractions of the leaf of *L. camara* Linn. gave positive reaction with Lieberman Burchard spray reagent. The same TLC profile as observed for Liebermann-Burchard was obtained with para- anisaldehyde, 10% sulphuric acid, and vanillin in sulphuric acid, all visibe under ordinary light. Triterpene saponins are the most valued in terms of pharmacology. They have anti-inflammatory, hypoglycaemic, and anti-cancer activity (Ji *et al.*

2011; Mukherjee *et al.* 2006; Sidhu & Oaekenful 1986). The hyopglycaemic activity of active fractions of extracts of L. camara Linn.leaf can be attributed to presence of triterpene glycosides.

Table 1. Phytochemical constituent of extracts of *lantana camara* Linn. leaf

Phytochemical constituents	Aqueous Ethanol extract	Petroleum Ether fraction	Chloroform fraction	Ethyl Acetate fraction	n-butanol Fraction	Aqueous fraction
Alkaloids	-	-	-	-	-	+
Anthraquinones (free)	-	-	-	-	-	-
(combined)	-	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+	+
Carbohydrates (reducing sugars)	+	-	-	-	+	+
Flavonoids	+	-	-	+	+	+
Saponins	+	-	-	+	+	+
Steroids/sterols	+	+	+	+	+	-
Tannins	+	-	-	+	+	+
Phlobatannins	-	-	-	-	-	-
Triterpenoids	-	-	-	-	+	+
Polyphenols	+	-	-	+	+	+

Key: - indicates Absent; + indicates Present.

Table 2. Effect of oral administration of seventy percent ethanol extract and fractions of *lantana camara* Linn. leaf on serum glucose level in normal and alloxan diabetic rats.

Animal grouping/Treatment	Biochemical Parameter Glucose (mmol/L)
Normal control	5.3 ± 0.5
Normal treated (Aqueous ethanol extract)	4.2 ± 1. 1[a]
Normal treated (Butanol fraction) Normal treated (Aqueous fraction) Diabetic control	4.2 ± 0. 3[a] 3.1 ± 1. 7[a] 20.5 ± 1.4
Diabetic treated (Aqueous ethanol extract)	15.2 ± 1. 3[b]
Diabetic treated (Butanol fraction)	13.7 ± 1. 9[b]
Diabetic treated (Aqueous fraction) Diabetic treated (Glibenlamide)	11.6 ± 1. 7[b] 09.3 ± 3. 1[b]

Values are mean ± S.D; n=5, a= p<0.05 compared with normal control, b= p<0.05 compared with diabetic control.

Table 3. Effect of oral administration of seventy percent ethanol extract and fractions of *Lantana camara* Linn. leaf on the lipid profile in normal and alloxan diabetic rats.

Animal Grouping	Total Cholesterol mmol/L	Triglyceride mmol/L	HDL – Cholesterol mmol/L	LDL – Cholesterol mmol/L
Diabetic Control	3.02 ± 0.4[a]	1.42 ± 0.2[a]	1.36 ± 0.2[a]	1.0 ± 0.1
Diabetic Treated (Aqueous ethanol extract)	2.86 ± 0.6	1.26 ± 0.2	1.16 ± 0.1	1.0 ± 0.0
Diabetic Treated (Butanol Fraction)	2.86 ± 0.1	1.24 ± 0.1	1.00 ± 0.2[b]	1.1 ± 0.1
Diabetic Treated (Aqueous Fraction)	2.58 ± 0.2	1.24 ± 0.3	0.98 ± 0.2[b]	1.0 ± 0.2
Normal control	2.22 ± 0.2	0.98 ± 0.1	0.92 ± 0.1	0.9 ± 0.0
Normal Treated (Aqueous ethanol Extract)	2.22 ± 0.3	0.94 ± 0.1	0.92 ± 0.2	0.9 ± 0.0
Normal Treated (Butanol Fraction)	2.15 ± 0.1	0.96 ± 0.1	0.80 ± 0.1	0.9 ± 0.1
Normal Treated (Aqueous fraction)	2.28 ± 0.2	0.82 ± 0.1	0.92 ± 0.1	0.9 ± 0.1

Values are mean ± S.D; n=5, a = p < 0.05 Compared with normal control,b = p < 0.05 Compared with diabetic control.

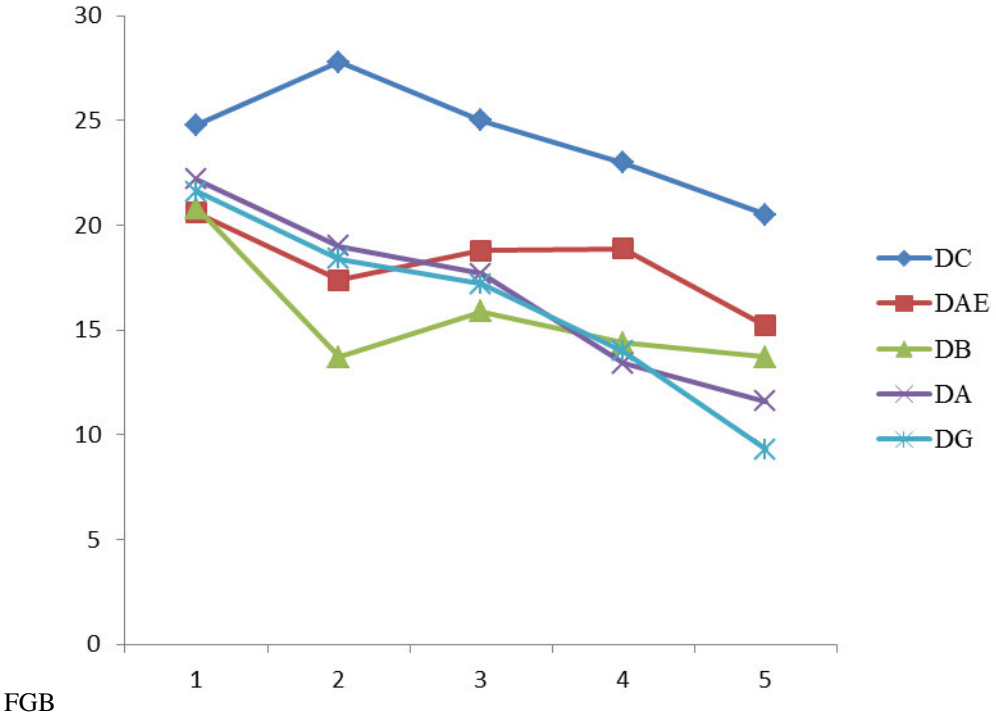

Figure 1. Glucose weekly monitoring showing pattern of hypoglycaemic action of extracts of *Lantana camara* Linn. leaf in diabetic rats. key: DC; diabetic control, DAE; diabetic seventy percent ethanol treated DB; diabetic n-butanol treated ,DA; diabetic aqueous fraction treated,DG;diabetic glibenclamide treated. FGB;fasting blood glucose in mmol/L.

Table 4. Chemical test for identification of isolated bioactive compound from aqueous fraction of *L.Camara* Linn. leaf.

Detection Spray	Observation	Inference
Alcoholic Aluminium Chloride (1%)	No reaction with compound. No yellow fluorescence seen under ultraviolet (UV) light	Flavonoid not detected
Alcoholic ferric Chloride (5%)	Reaction with compound, faint pinkisk red coloured developed whose intensity increased with time	Trace of phenolics detected
Borntrager Reagent	No reaction with compound	Anthraquinones and coumarins not detected
2,4 – Dinitrophenyl hydrazine	No instant reaction occurred. After heating, reaction occurred with compound. Red colouration observed	Aldehyde/ketone group detected. A sugar molecule may be present
2,2-Dinitrophenyl, 1-picrylhydrazyl	No instant reaction .After few minutes reaction occurred. purple colour observed which disappeared with time, leaving a white spot	Antioxidant activity may be present
Dragendorff's Reagent	No reaction with compound	Alkaloids not detected
Liebermann- Burchard	Instant reaction occurred. Compound turned blue on spraying. After heating, Compound turned purple/violet.	Triterpene detected/present
Para-anisaldehyde	Instant reaction occurred. Compound turned blue on spraying. After heating, compound turned purple/violet	Terpenes, Sugars, Phenols ,or Steroids present
Sulphuric acid (10%)	Instant reaction occurred. Compound turned blue on spraying. After heating, compound turned purple/violet	Single compound confirmed. Reaction same with para-anisaldehyde spray
Vanillin in sulphuric acid	Instant reaction occurred. Compound turned blue on spraying. After heating, compound turned purple/violet	Terpenes or Steroids present

Table 5. Chemical test for identification of isolated bioactive compound from butanol fraction of *L.camara* Linn. leaf.

Detection Spray	Observation	Inference
Alcoholic Aluminum Chloride (1%)	No reaction with compound. No yellow fluorescence seen under ultraviolet (UV) light	Flavonoid not detected
Alcoholic ferric Chloride(5%)	No reaction with compound	Phenolics not detected
Borntrager Reagent	No reaction with compound	Anthraquinones and coumarins not detected
2,4 – Dintrophenyl hydrazine	No instant reaction. After heating, reaction occurred with compound. Red colouration observed	Aldehyde/ketone group detected. A sugar molecule may be present.
2,2-Dinirophenyl, 1-Picrylhyrazyl	No instant reaction.After few minutes reaction occurred. purple colour observed which disappeared with time,leaving white spot	Antioxidant activity may be present
Dragendorff's Reagent	No reaction with compound	Alkaloids not detected
Liebermann – Burchard	Compound turned purple/violet after heating	Triterpene detected/present
Para- anisaldehyde	Compound turned green after heating	Terpenes, sugars, phenols, steroids may be present
Sulphuric Acid (10%)	Compound turned green after heating	Single compound confirmed. Reaction same with para-anisaldehyde spray
Vanillin in Sulphuric acid	Compound turned purple/violet after heating	Terpenes and other compounds may be present

4. Conclusion

This study provided evidence of antidiabetic action of extract and fractions of *L.camara* Linn.leaf. Triterpenes are possibly responsible for the hypoglycaemic activity. Discovery of new drugs to fight incurable diseases such as diabetes mellitus is an on-going area of research by scientist worldwide. Many plant-derived triterpenes have been reported to exert beneficial effects in metabolic disorders.

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