academicJournals

Vol. 10(5), pp. 54-58, 3 February, 2016
DOI: 10.5897/JMPR2014.5704
Article Number: AAF972D57082
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Antioxidative and anti-hyperglycaemic effect of *calotropis procera* in alloxan induced diabetic rats

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Received 25 November, 2014; Accepted 15 December, 2015

This study investigated the anti-hyperglycaemic and antioxidative properties of *Calotropis procera* (Tumfafiya) leaf extract on an alloxan induced diabetic rats. The rats were fed on commercial diet and grouped into 4. Group1 as diabetic treated with leave extract (DTL), group 2 diabetic on normal diet (DNT), group 3 as non diabetic on normal diet (NDNT) and group 4 as diabetic treated with chlorpropamide (DCP). The successful diabetic induction was achieved by intra-peritoneal injection of 180 mg/kg body weight with alloxan. The sustenance and severity of diabetes was assessed using fasting blood glucose for one week. The plant extract was then administered to the induced rats at 100 mg/kg body for four weeks after which a serum glucose level was assessed at weakly intervals. Malondialdehyde was also measured for anti-oxidative effect. The results indicated that the extracts possessed significant hypoglycemic effect on the DTL group, with mean glucose of 5.9 ± 0.2 mmol/L compared to the DNT control group with a mean glucose of 15.5 ± 0.2 mmol/L ($p < 0.05$). A raised malondialdehyde was also observed among the DNT (23.2 ± 3.5) as against DTL group (12.5 ± 0.5) mmol/L, ($p < 0.05$). In conclusion, it shows that C*alotropis procera* methanolic leaf extract have a potential hypoglycemic effect in alloxan induced diabetic rats and also with antioxidant property.

Key words: *Colotropis procera*, anti-hyperglycaemic agent, antioxidant, alloxan, diabetes mellitus, chlopropamide.

INTRODUCTION

Diabetes mellitus is a metabolism disorder characterized by inappropriate hyperglycemia (Ceriello, 2005), caused by a relative or absolute deficiency of insulin or by resistance to the action of insulin at the cellular level (Bakari and Narayan, 2003; Fasanmade et al, 2008). It is the most common endocrine disorder, affecting as many as 200 million people worldwide (Debra, 1991; Brown et al., 2003). The clinical consequences of the syndrome include blindness, heart and blood vessel disease, stroke, kidney failure, amputation and nerve damage and with up to 80% of death in people with diabetes caused by cardiovascular in the developed world (Cariello and Motz, 2004). *Colotropis procera* belongs to the family Asclepiadaceae with various nomenclatures as Sodom

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apple, auricular tree, deadsea apple, swallow-worth, giant-milk weed, madar, mudar, rubber bush, small crown flower, sodom's-milk weed, from different ethnic or geographical locations (Ansari and Ali, 1999; Aliyu et al., 2006). In Hausaland, it is called Tunfafiya or Bambambele and found on dry savannah and other arid areas, mostly anthropogenic occurring around villages (Aliyu et al., 2006). *C. procera* is reported to contain cardenolides besides steroids and triterpenes (Ansari and Ali, 1999; Shrivastava et al, 2013). From the hexane-insoluble fraction of this medically important plant, a new cardenolide named proceragenin was isolated. Pharmacological screening of proceragenin revealed to poses antibacterial and anti-aggregating activities (Akhtar, 1984; Uddin et al, 2012).

There is a reservoir of basic information on the ethno medicinal uses of *C. procera* in the treatment of headache, catarrh, conjunctivitis, skin disease and wound (Vishwa, 2004). Other uses include treatment against asthma, cold, cough, labor, leprosy, parturition, small pox, sore, splenitis, purgative, fatality, fumitory, dyspepsia (Duke, 1994; Kawo et al., 2013). However, there is paucity of information as an anti-diabetic agent though used by herbalist in Hausaland, in the management of diabetic patients. The present research was conducted to study the potential hypoglycaemic and anti-oxidative activities of the leaf extract of *C. procera* in alloxan induced diabetes rats. An extension was also made to provide an introductory approach for the evaluation of its traditional preparation in order to scientifically validate the therapeutic preparation of this plant in the control of diabetes in our locality.

MATERIALS and METHODS

All the chemicals and reagents used are of analytical grade and were obtained from Sigma-Aldrich (St Louis, USA).

Plant collection

The plants of *C. procera* as a whole were collected from natural populations of the Bayero University, Kano. The plant was authenticated and identified at the Botanical Unit of the Department of Biological Science. The plant material was shade dried at room temperature for 10 days, coarsely powdered with the help of a hand grinding mill, and the powder was passed through sieve.

Phytochemical sreening

Phytochemical screening of the leaf extracts was carried out following the standard methods prescribed by Sofowara in 1993 and Trease and Evans, 1989 evaluate the presence of various chemical constituents in ethanolic leaf extracts.

Experimental animals

White albino rats were obtained from the animal house of Physiology Department, Bayero University Kano, where the animals were kept for the period of study.

Plant extract preparation

C. procera leaf was collected and dried under shade for 7 days. The dried leaves were grounded into powder manually and processed to mill. About 750 g of the powdered leaf was added to a container with 1,500 ml methanol and allowed to stand for 8 days with occasional swirling and shaking. The content was then evaporated using rotator evaporator for a solid leaf extract.

Extract administration

From the dried extract, one teaspoon of the powdered leaf extract weighing 8.98 g was mixed with the animal feed weighing 100 g. The mixed animal feed with the leaf extract was fed to the animals on daily basis for the period of four consecutive weeks. The treatment was stopped and the rats were sacrificed. During the entire four weeks of the experiment, fasting blood glucose of the rats was monitored at an interval of one week.

Induction of diabetes

All animals were fed on normal diet and left to acclimatize for a week. Diabetes was induced by a single injection of 100 mg/kg body weight alloxan monohydrate freshly prepared, through intraperitoneal (IP) route to fasting rats for at least 10 h. Blood glucose level were measured prior to induction and after the induction.

Animal feed

Commercially prepared rats feed (grower mash) was obtained from PS mandrides plc, Kano, Nigeria. The composition of the feed is given below:

Crude protein	15.50%
Crude fiber	7.40%
Calcium	6.59%
Phosphorus	4.85%
Metabolized energy cal/kg	2500k

Experimental design

A total of 12 Wistar (albino) rats were randomly grouped into four (4) groups as follows:

Group 1: Alloxan- induced diabetic rats administered treated with mixed feed (DLT).
Group 2: Negative control group fed with normal animal feed (NDNT).
Group 3: Positive control group fed with normal animal feed (DNT).
Group 4: Diabetic treated with Chlorpropamide (DTCP).

Sample collection

Weekly samples for the estimation of glucose was obtained aseptically and treated accordingly to guard against lost on storage. At the end of the four weeks, the rats were sacrificed by surgical dislocation of the neck and the blood was collected into test tubes and centrifuged to get the serum for the biochemical analysis. The Serum was used for the estimation of glucose (fasting blood sugar) and malondialdehyde (MDA).

Table 1. Phytochemical screening of crude leaf extract *Calotropis procera*

Chemical constituents	Methanol
Alkaloids	+
Terpenoids	+
Flavonoids	+
Anthraquinones	-
Tannins	+
Saponins	+
Glycosides	+
Reducing sugars	-
Steroids	+

Table 2. Fasting blood sugar (mmol/l) of alloxan induced diabetic rats treated with *calotropis procera* leave extracts.

Parameter	Serum glucose level (mmol/L) at induction and post treatment			
	Leaf (Ext)	Controls		CP (8.4 mg/kg)
Week	DTL	DNT	NDNT	DTCP
1 (ind)	13.95±1.26	**1**2.85±0.36	4.32±0.22	12.90±0.20
2	10.93±0.47	13.79±0.31	4.16±0.14	10.44±0.08
3	8.07±0.48	13.12±0.16	5.48±0.32	8.99±0.09
4	6.42±0.58	14.29±0.32	3.79±0.08	8.27±0.13
5	**5**.91±0.17	14.51±0.18	4.33±0.13	6.06±0.07

DTL=diabetic treated; DNT=Diabetic non treated; NDNT=Non-diabetic non treated; ind=induction week. Values are mean ± standard error of mean (n = 12); CP= Chlorpropamide.

Biochemical analysis

Serum glucose estimation using glucose oxidase method

Fasting blood glucose was estimated by glucose oxidase method according to manufacturer's instruction (Erba diagnostics, Mannhem, Gmbh Germany).

Determination of malondialdehyde (MDA) (Gutterridge and Wilkins, 1982)

Malondialdehyde is an organic compound with the formula $CH_2(CHO)_2$. The structure of this species is more complex than this formula suggest. The reactive species occurs naturally and is a marker for oxidative stress. Lipid peroxidation generates peroxide intermediates which upon cleavage releases MDA a product which react with thiobarbituric acid (TBA). The product of the reaction is a colored complex which absorbs light at 532 nm.

RESULTS

Phytochemical screening

Phyto-chemical screening of the crude extract of *C. procera* leaf was carried out to ascertain the presence of its bioactive constituents utilizing standard methods.

Mean serum glucose level during extract administration

There was a decrease in mean serum glucose level (mmol/L) of the rats from the first to the fourth week of plant extract administration. Tables 1 to 3.

Mean malonyldialdehyde concentrations among alloxan induced diabetic and controls

Malonyldialdehyde concentration was estimated at the end of four weeks post induction. As compared, there was an observed significant increase among DNT group than any other group in the study.

DISCUSSION

Diabetic mellitus is a metabolic disease associated with

Table 3. Malonyldialdehyde (mmol/L) concentrations among alloxan induced diabetic rats and controls.

Groups	Malondialdehyde (mmol/L)
DT L	12.5±0.46†
DNT	23.2±3.46
DTCP	25.2±5.65
NDNT	8.2±0.55

DTL=diabetic treated; NDNT=non-diabetic non-treated; DNT=diabetic untreated; DTCP=Diabetic treated with Chlorpropamide. Values are mean ± standard error of mean (n = 12), †=significant.

impaired glucose metabolism which in effect alters intermediary metabolism of lipids, of which most of the complications of the diabetic state are initiated by the generation of free radicals (Kumar et al., 2005). Despite immense technological advancement in modern medicine, many people in developing countries still depend on traditional healing practices and medicinal plants for their daily health care needs. There exists a good virtue to intensify researches into plants for medicine, especially those that will reduce the burden in serious disorders, such as diabetes mellitus. In trado-medical practice by herbalists, it is believed that *C. procera* is used locally for the treatment of diabetes mellitus. Phytochemical studies unveil that extract of *C. procera* contains terpenoids, steroids, glycosides, saponins, alkaloids and flavonoids similar to the work reported by Verma et al. in 2013 and Kawo et al. in 2013; Shrivastava et al 2013. Minerals such as magnesium, manganese, zinc, iron, phosphorous, copper and calcium among others were reported in the work of Vishwa (2014). The study as highlighted by the presence of many secondary metabolites provides an overview of the different classes of molecules present that have led to their pharmacological activities. There was observed in this research work, a significant reduction in mean serum glucose of the rat treated with the plant leaf extract (5.91 ± 0.17) when compared with the mean serum glucose of the diabetic control rats (14.51 ± 0.18) ($p < 0.05$). The hypoglycemic effect of the plant extracts was maintained throughout the period of administration. The detection of flavonoids in the extract might be a pivot to the blood glucose lowering property. Flavonoid is believed to inhibit glucose-6-phophatase activity in a liver, thereby suppressing gluconeogenesis and glycogenolysis and consequently reduces the hyperglycaemia. This can therefore be the reason behind the progressive decrease in the antidiabetogenic effect of the extract among DLT group from induction down the 4th week. Some plants extracts with similar elemental and organic constituents have same biochemical influence on glycaemic control as reported by Rajendran et al. in 2007 on *Aloe vera* with a wide range of medicinal applications such as lowering blood sugar in diabetes, ulcer curative effect, stimulating immune response against cancer etc. These effects are being attributed to the role of inorganic elements like zinc, iron, copper, magnesium and manganese in collaboration with flavonoids and alkaloids in the improvement of impaired glucose tolerance. The concentration of the MDA across the set groups were significantly higher in DNT (23.2 ± 3.46) and DTCP (25.2 ± 5.65) compared to that of DTL (12.5 ± 0.46) and NDNT (8.2 ± 0.55) ($p < 0.005$) groups statistically.

Malondialdehyde is a degradative product of peroxidation of polyunsaturated fatty acids (PUPA) in the cells membrane (Ohakawa et al., 1979). The presence of MDA in circulation indicates oxidative stress which has been reported as one of the underlying cause of hyperglycaemia. *C. procera* possesses potent co-factors among the minerals reported by Vishwa in 2014, for a number of antioxidants by increasing levels of endogenous antioxidants, viz. superoxide dismutase, catalase and glutathione and bring down the level of thiobarbituratic acid-reactive substance and anti-hyperglycemic effects.

CONCLUSION

Significant number of researches have shown the presence of a wide variety of bioactive compounds in the leaf, stem and roots of medicinal plants including *C. procera* that have beneficial effects on human health. Considering the fact that diabetic mellitus have reached epidemic proportions in many countries with increase in socio-economic burden, it will be important to have an alternative that will help in prevention and treatment of this disease. The studies using *C. procera* as a medicinal plant bring information that may provide validation for its medicinal uses. A more extensive research involving clinical trials is requested so that it could be recommended for prevention and as an adjuvant in the treatment of diabetic mellitus and its complication.

RECOMMENDATIONS

As part of the efforts in evaluating the anti-hyperglycemic effects of leaf extract of *C. procera,* It is recommended that the histology of the liver cell be carried out so as to have the physical observation of the toxicity in liver. Also, the antioxidant effect should be assayed by using other oxidative stress marker, such as super oxide dismutase, glutathione peroxidase, etc to confirm the antioxidant effect.

Conflict of interest

The authors have not declared any conflict of interest.

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