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ANTIDIABETIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *CROTON ZAMBESICUS* MUELL.(THUNDER PLANT) IN ALLOXAN DIABETIC RATS

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Abstract

Antidiabetic activity of ethanolic leaf extract of *Croton zambesicus* Muell. Arg. was evaluated using alloxan-induced (150mg/kg) hyperglycaemic rats. The activity of the ethanolic leaf extract was compared with that of a reference drug Chlorpropamide. The Blood Glucose Levels were measured using glucometer. The extract produced a significant ($P<0.01$) reduction in BGL after a single dose of the extract and in prolonged treatment (for 7 days). The antidiabetic activity was comparable to that of the reference drug-chlorpropamide.

Key words: *Croton zambesicus*, antidiabetic, alloxan

Introduction

Croton zambesicus Muell Arg. (Euphorbiaceace) (syn *C. amabilis* Muell. Arg. *C. gratissimus* Buch) is an ornamental tree grown in villages and towns in Nigeria. It is a Guineo-Congolese species widely spread in tropical Africa. The leaf decoction is used in Benin as anti hypertensive and anti-microbial (urinary infections) and to treat fever associated with malaria (Adjanohoun et al., 1989) and in parts of Nigeria as antidiabetic and malarial remedy. Block *et al.*, (2002) reported that ent-trachylobane diterpene, isolated from dichloromethane extract of the leaves has cytotoxic activity on Hela cells. Studies have reported on the antimicrobial properties of the leaf and stem (Abo et al., 1999). The essential oil found in the leaves contain P-cymene, linalool and beta-caryophyllene (Menut et al., 1995). The constituent of the essential oil also found in the flowering tops are pinene, limonene, methol, carvone, thymol, alpha – humulene and ceisnerolidol (Mekkawi, 1985). The ethanolic leaf extract has been reported to possess antiplasmodial activity (Okokon et al., 2005). Although a number of studies have been carried out on this plant, there is no scientific report on the hypoglycaemic activity of this plant grown in Nigeria. We, therefore investigated the above property of the plant to confirm it's use traditionally.

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Materials and Methods

Preparation of plant extract

The leaves of *C. zambesius* Muell. Arg. (Euphorbiaceae) were collected in November 2004 at Uyo area of Akwa Ibom State Nigeria and authenticated by Dr. Margaret Bassey a taxonomist in the department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimen number of the plant is FPUU 209. The fresh leaves (2kg) of the plant were dried on a laboratory table for 8 days and reduced to power. The powder 100g was macerated in 95% ethanol (300ml) for 72 hours. The liquid extract obtained was concentrated *in vacuo* at 40^0C. The yield was 3.77%. The extract was stored in a refrigerator at 4^0C until used.

Animals

Albino wistar rats (139-220g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

Determination of LD_{50}

The LD_{50} of the extract was estimated using swiss albino mice by intraperitoneal (i.p. route using the method of Lorke (1983).

Induction of diabetes

Alloxan monohydrate (BDH) 150mg/kg body weight was injected intraperitoneally to induce hyperglycemia (Yanardag and Colak, 1998). The experimental animals were fasted for 18h before alloxan injection. After one hour of alloxan administration the animals were fed *ad libitum*.

Evaluation of antidiabetic activity

The Blood Glucose Level (BGL) was monitored after alloxanisation in blood samples collected by amputation of the tail tip under mild anaesthesia. The blood was dropped on the dextrostix reagent pad. The strip was inserted into microcroprocessor digital blood glucometer and the reading were noted (WHO, 1980). After 72 hours rats having BGL beyond 150mg/dl of blood were selected for the study and divided into five groups of five animals each. The extract 100, 150 and 200 mg/kg were orally administered to respective groups of animals. The reference drug chlorpropamide (100mg/kg) and normal saline were also administered orally to two groups of animals representing reference and control groups respectively. The above treatments were carried out in each group of animals for seven consecutive days. The blood glucose level was monitored after 1, 3, 6 and 12 h of administration of a single dose of the extract (for acute study) and at the end of 1, 2, 3 and 7 days (prolonged treatment).

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Statistical analysis

Data obtained were analysed using student's t-test to determine the statistical significance of the change in BGL. $P < 0.05$ was considered significant.

Results

Acute toxicity

The extract (100-200mg/kg) produced physical signs of toxicity ranging from decreased motor activity, writhing, decreased respiratory rate, body and limb tone to death. The intensities of all these effects were proportional to the dose administered. The LD_{50} of the extract in mice was 1400 ± 148 mg/kg.

TAble 1: Effect of *C. zambesicus on blood glucose levels of alloxan diabetic rats after a single dos**e.***

	Dose	Blood glucose level (Mg/dl) (mean ± SEM)				
Drug	Mg/kg	Initial	1h	3h	6h	12h
Control	-	253.4±61	260.3±9.53	262.5±10.15	260.2±1.17	256.9±4.67
Extract	200	249.5±6.75	128.0±5.50*	111.0±2.00*	99.3±6.03*	92.9±8.51*
	150	262.3±4.00	146.0±12.50 *	118.0±8.00*	110.4±3.94 *	103.1±3.04 *
	100	248.5±10.2 5	169.0±16.75	124.1±3.15*	119.2±7.81 *	107.3±2.81 *
Chlorpropamide	100	258.0±5.00	110.5±10.75 *	161.5±8.75*	91.5±3.00*	86.5±3.77*

n = 5, * P < 0.01 Vs control

Table 2: Efect of *c. zambesicus on blood glucose levels of alloxan diabetic rats after prolonged treatment*

	Dose	Blood glucose level (Mg/dl) (mean ± SEM)				
Drug	Mg/kg	Initial	1st day	2nd day	3rd day	7th day
Control	-	253.4±6.61	247.2±9.57	245.7±8.12	252.1±5.14	243.0±5.01
Extract	200	249.5±6.75	94.6±13.81*	70.3±12.71*	64.3±13.43 *	63.7±2.18*
	150	262.3±4.00	100.5±7.28*	77.9±9.05*	75.5±4.13*	73.6±1.17
	100	248.5±10.2 5	117.5±8.39*	81.3±10.38*	79.6±8.41*	75.1±0.91*
Chlorpropamide	100	258.0±5.00	87.6±11.06*	71.4±3.52*	66.4±3.92*	61.3±6.80*

n = 5, * P < 0.01 Vs control

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Hypoglycaemic activity

Administration of ethanolic leaf extract of *C. zambesicus* (100-200mg/kgg) to alloxan –diabetic rats produced a significant ($p<0.01$) reduction in BGL of the diabetic rats in a dose dependent fashion after a single dose of the extract and in prolonged treatment (7 days) compared to control group. The hypoglycaemic activity of the extract was sustained throughout the monitoring period. At 12 hours after a single dose of the extract about 59.6+3.04% reduction in the BGL of the alloxanized rats was recorded almost comparable to that of the reference drug (chlorpropamide) (Table 1). The extract (100-200mg/kg) produced a sustained significant ($P<0.01$) hypoglycaemic effect during prolonged treatment (7days) and this was comparable to that produced by the reference drug (Table 2).

Discussion

A number of plants have been used traditionally to treat diabetes and some have been proven scientifically and reported to have hypoglycaemic effect. These studies on hypoglycaemic activity of plants have identified compounds like polysaccharides (Tomoda et al, 1985), flavonoids (Schimizu et al, 1984), terpenoids and Tannins (Reher *et al*, 1991) steriods (Ivorra *et al*, 1989), glycoprotein (Hikino et al, 1989), polypeptides (Khana and Jain, 1981) and alkaloids (Karawya and Wahab,1984) to be responsible for this action. *C. zambesicus* ethanolic leaf extract have been reported to contain saponins, alkaloids, terpenes, flavonoids and cardiac glycosides among others (Okokon et al, 2004).The observed hypoglycaemic effects of this plant could have resulted from the combined activity of these compounds present in the leaf extract. Sulphonylureas cause hypoglycemia by stimulating insulin releases from pancreatic beta cells. The effects of the sulphonylureas are initiated by binding and blocking an ATP- dependent potassium channels present in the beta cell membrane in the pancreatic islet cells(Aguillar-Bryan et al, 1995).Moreso, studies have reported on hypoglycaemic activty of *Croton cajucara*, a species in the same genus.trans- Dehydrocrotonin, a nor – clerodane diterpene isolated from the plant was found to exert significant hypoglycaemic activity in alloxan induced diabetic rats(Farias et al,1997).Isolation from the leaves of *Croton zambeslcus* of four diterpinoids compounds have been reported(Block *et al*,2002;Block *et al*,2004).Also,labdane,clerodane and trachylobane diterpenes have been identified in the stem bark of *Croton zambesicus* (Ngadjui et al,2002).This further points to the involvement of the diterpenes present in the leaves in this activity. Sulphonylureas compounds are potent in mild alloxan-induced diabetes and inactive in intense alloxan diabetes where nearly all beta cells have been destroyed (Yallow et al, 1960). The observed reduction in blood glucose level of hypergylcaemic animals by chlorpropamide in our study portrays an insevere state of diabetes. Moreover, the comparable pattern of hypoglycaemic activity of the extract under study with that of the reference drug, chlorpropamide, demonstrated a possible similarity in their mechanism of action to increase insulin secretion.

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Conclusion

This observation confirms the use of this plant in ethnomedical practice for diabetes management. It also warrant further investigation to isolate and identify the hypoglycaemic principles in this plant so as to elucidate it's mode of action.

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References

1. Abo, K. A.; Ogunleye, A.; Ashidi, J. S. (1999), Antimicrobial Potential of Spondias Mombin, *Croton Zambesicus,* and *Zygotoitonia crocea.* Phytother. Res. **13 (6):** 494 – 497.
2. Adjanohaun, E. J., Adjakidje, V. and de Souza, S. (1989), Contribution aux Etudes Ethnobotaniques et floristiques en Republic Populaire du Benin,Vol.1.Agence de cooperation Culturelle et Technique,Paris,pp 245
3. Aguilar-Bryan, L. Nicholas, C. G., Wescheler S. W., Clementivj, P., Body A. E., Gonzalez, G., Herreraspsa H, Nguy K, Bryan J. and Nelson,D. A. (1995), Cloning of the β-cell high-sulfonylurea receptor: a regulator of insulin secretion. Science **268:**423-426.
4. Block S, Stevigny C., De Pauw-Gillet M. C., de Hoffman E, Liabres G. Adjakidje V. and Quertin-Leclerg J. (2002) enttrachyloban – 3-01, a New Cytotoxic Diterpene from *Croton zambesicus*. Planta Medica **68:** 647-648.
5. Block, S., Chiara, B.,, Bernard, T., Meervelt, L. V., Rozenberg, R., Jiwan, J. H., Llabres, G., De Pauw-Gillet, M. C. and Leclerg, J. Q. (2004). Diterpenes from the leaves of *Croton zambesicus.* Phytochemistry.**65(8):**1165-1171.
6. Farias, R. A. F., Rao, V. S. N., Viana, G. S. B., Silveira, E. R., Maciel, M. A. M. Pinto, A. C. (1997). Hypoglycaemic effect of trans- Dehydrocrotonin, a Nor-clerodane diterpenes from *Croton cajucara.*. Planta Medica.**63:**558-560.
7. Hikino, H., Kobayashi, M., Suzuki, Y. and Konno, C. (1989). Mechanism of hypoglycemic activity of aconitan A, a glycan from *Acanitum carmichaeli* roots. J. Ethnopharmacol. **25:** 295-304.
8. Ivorra, M. D., Paya, M., and Villar, A. (1989), A Review of natural Products and Plants as potential antidiabetic drugs. J. Ethnopharmacol. **27:**243-275.
9. Karawya, M. S. and Wahab, S. A. (1984). Diphenylamine, an antihyperglycemic agent from onion and tea. J. Nat. Product **47:** 775-780.
10. Khanna, P. and Jain, S. C. (1981). Hypoglycaemic activity of polypeptide-p from a plant source. J. Nat. Product. **44:** 648 – 655.
11. Lorke, D. (1983). A new approach to practical acute toxicity testing. Archieves Toxicology **54:** 275-287.
12. Mekkawi, A. C. (1985), The essential oil of *Croton zambesicus* Fitoterapia **56(3):** 181-183.

13. Menut, C, Lamaty, G, Bessiere J. M., Suleiman A. M., Fendro, L., Maidou, E. and Denamgania J. (1985), Aromatic Plants of Tropical Central Africa. J.Essential Oil Res.. **7(4):** 419-422.
14. Ngadjui, B. T., Abengaz, B. M., Keumedjio, F., Folefoc, G. N. and Kapche G. W. (2002) Diterpenoids from the stem bark of *Croton zambesicus.*Phytochemistry.**60:**345- 349.
15. Okokon, J. E., Ofodum, K. C., Ajibesin, K. K, Danlandi, B. and Gamaniel, K. S. (2005). Preliminary Pharmacological screening and Evaluation of antiplasmadial activity of *Croton zambesicus* against *P. berghii berghei* infection in mice. Ind. *J.* Pharmacol. **37(4):** 243- 246.
16. Reher, G., Slijepcevic, M. and Krans, L. (1991), Hypoglycemic activity of triterpenes and tannins from *Sarcopoterium spinosum* and two *Sanguisorba* Species. *Planta Med.* 57: A57-A58.
17. Schimizu,M., Ito, T.,Rshima,S., Mayashi,T., Arisawa,M., Morita, , Kurokowa,S. Ito Hasimato Y. (1984). Inhibition of lens aldose reductase by flavonoids Phytochemistry **23:** 1885 – 1888.
18. Tomoda,M, Shimada,K. Konno C., Hikino, H., (1985), Structure of Panaxan B., A. Hypoglycaemic glycan of Panax ginseng roots. Phytochemistry **24:** 2431-2433.
19. Yallow,R. S., Black H, Villazan, M., Berson S. A. (1960).Comparison of Plasma Insulin levels following administration of tolbutamide and glucose, Diabetes **9:** 356 – 362.
20. Yanarday, R. and Colak H. (1998), Effect of Chard (Beta vulgaris L. Varcicla) on blood glucose levels in Normal and alloxan-induced diabetic rabbits. Pharm. Pharmacol. Comm. **4:** 309 - 311.