International Scholars Journals

African Journal of Medical Case Reports Vol. 6 (1), pp. 001-004, January, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

A comparative study on the anti-diabetic activity of extracts of some Algerian and Sudanese plants

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Accepted 20 October, 2017

The present comparative study was undertaken to determine the hypoglycemic effect of 96% ethanolic extracts of *Eucalyptus globulus* (Myrtaceae) leaves, *Salvia officinalis* (Lamiaceae) leaves growing in Algeria and *Guiera senegalensis* (Combretaceae) leaves growing in Sudan in glucose loaded albino rats and to assess their toxicity. Preliminary phytochemical screening of leaf extracts of the three plants revealed the presence of carbohydrates, tannins, flavonoids, sterols and triterpenes, alkaloids and terpenoids. Graded doses of the aqueous ethanolic leaf extracts of the three plants, 200 to 400 mg/kg were separately administered orally to groups of glucose loaded rats. The hypoglycemic effect of the extracts was compared to glibenclamide 10 mg/kg in fasted normal rats. Following the treatment, relatively moderate to high doses of the three extracts produced a dose-dependent significant reduction in blood glucose levels which was most significant at the dose 400 mg/kg. The median lethal concentration LC_{50} in brine shrimps was 55.95 µg/ml for *E. globulus*; 37.20 µg/ml for *S. officinalis* and 26.94 µg/ml for *G. senegalensis* compared to a standard antitumor drug Etopside. The results clearly demonstrated that the 96% alcoholic leaf extracts of the three plants had a significant blood-glucose lowering potential in glucose loaded rats with minimum toxicity.

Key words: Sudan, Algeria, leaf extracts, *Eucalyptus globulus*, *Salvia officinalis*, *Guiera senegalensis*, hypoglycemic effect, toxicity.

INTRODUCTION

Uncontrolled diabetes leads to several micro-vascular (neuropathy, nephropathy, retinopathy) and macro-vascular (atheroma) complications that affect many organs of the body. Diet plays an important role in the management of diabetes mellitus and the health-beneficial effects of dietary fibers and antioxidants de-rived from plant food sources have been extensively studied (Borries, 1996; Alison and Peter, 1998; Alves et al., 2000; Kivak and Tansel, 2001).

The increasing prevalence of the disease worldwide is a result of sedentary life-style. Many side effects that result from the synthetic hypoglycemic agents led to an increase in the search for new sources in treatment of diabetes. Plants used traditionally as medicines constitute potentially useful resources of new drugs for treatment and control of diseases including diabetes (Akah and Okafor, 1995; Silva et al., 1997; Konuklugil et al., 1997; Alireza and Mohammad, 2009). Good documentation of results and comprehensive data concerning their toxicity is essential to solve problems associated with current drugs used in the community. Several studies have been conducted in search for new hypoglycemic agents from plants and to assess their activity and toxicity in experimental animals and human beings (Cristova et al., 2006; Azza et al., 2009).

The present paper reports results to assess the hypoglycemic activity of ethanolic leaf extracts of *Eucalyptus globulus* (Myrtaceae) and *Salvia officinalis* (Lamiaceae) of Algerian origin, and *Guiera senegalensis* (Combretaceae) of Sudanese origin compared to the standard reference glibenclamide. Phytochemical

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screening of the bioactive leaf extracts of the three plants and their acute toxicity using the brine shrimp lethality test, are also reported in the present paper.

MATERIALS AND METHODS

Plant material

The study was undertaken at the Faculty of Pharmacy, University of Medical Sciences and Technology, Khartoum, Sudan, during November, 2010 to June, 2011. *E. globulus* and *S. officinalis* leaf samples were brought from Algeria during October, 2010 and *G. senegalensis* leaf sample from Khartoum local market, Sudan. The plants were identified and authenticated at the Medicinal and Aromatic Plants Research Institute, the National Centre for Research, Khartoum, Sudan and voucher specimens were deposited at the institute herbarium (Gamal et al., 1998).

Preparation of extracts

Dried leaves of the three plants were ground to powder and samples of 60 g were extracted in a Soxhlet apparatus with 96% ethanol. The extracts were concentrated under vacuum to dryness and residues were weighed to calculate extractable materials of the three samples with 96% ethanol. The extracts were used in phytochemical screening tests and assessment of toxicity and hypoglycemic effect on experimental rats.

Phytochemical screening

The leaf extracts of the three plants were phytochemically screened according to the method of Alves et al. (2000) and Saadabi and Ayoub (2009), and results of presence or absence of certain metabolites were reported.

Determination of hypoglycemic activity

Experimental albino rats of both sexes weighing from 120 to 250 g were used in the experiments and five groups each of six rats were fasted for 18 h prior to the tests. All groups were given 50% glucose solution at a dose of 10 mg/kg body weight. The first group was given *G. senegalensis* ethanolic leaf extract at dose of 200 mg/kg body weight, the second and third groups were given *E. globulus* and *S. officinalis* ethanolic leaf extracts at the same dose, respectively. The fourth group (positive control group) was given the standard drug glibenclamide at a dose of 10 mg/kg body weight, while the fifth group (negative control group) was given distilled water at a dose of 10 mg/kg body weight.

The plasma glucose level was monitored at 0, 2 and 4 h intervals. The experiments were repeated again with 400 mg/kg extracts (Alison and Peter, 1998; Cristova et al., 2006; Alireza and Mohammad, 2009).

Brine shrimp lethality assay

A half-filled hatching rectangular tray 22 × 32 cm with brine solution was sprinkled with 50 mg eggs of brine shrimps and incubated at 37°C for 48 h.

The samples of ethanolic leaf extracts of *G. senegalensis*, *E. globulus* and *S. officinalis* were prepared by dissolving of 20 mg of the sample in 2 ml of ethanol and 5, 50 and 500 µl of each sample were transferred into vials (3 vials / concentration) to be 10, 100, 1000 µg/ml, respectively, and the solvents were allowed to evaporate.

After two days of hatching and maturation, 10 larvae/vial were placed using Pasteur pipette and volumes were adjusted to 5 ml with sea water followed by incubation at 25 to 27°C for 24 h under illumination. Negative and positive control vials were filled with pure solvent and reference cytotoxic drug (etopside), respectively (Mayer et al., 1982; Caballo et al., 2002).

Data were analyzed with Finney computer programme to determine the LC_{50} values with 95% confidence intervals and using the formula:

$$\%Death = \frac{Sum\ of\ \ dead\ \ shrimps}{Sum\ of\ \ survived\ \ shrimps} \times 100$$

RESULTS AND DISCUSSION

The prepared 96% ethanolic leaf extracts of *E. globulus* and *S. officinalis* of the Algerian origin and *G. senegalensis* of the Sudanese origin were in the range of 3 to 4%, and were used in phytochemical screening and in assessment of hypoglycemic activity. The results of phytochemical screening of the three plants are presented in Table 1 and showed similar composition in the presence of flavonoids, tannins, carbohydrates, sterols and tritepenes, and alkaloids and saponins. The presence of volatile oils in the three plants was determined by hydrodistillation. Anthracenes, coumarins, cardiac glycosides and reducing sugars were not detected in the three samples.

The effects of the 96% ethanolic leaf extracts on hyperglycemic rats were investigated by experiments on rats of both sexes fasted for 18 h in five groups each of six rats of known weight (120 to 250 g) and results were reported in Table 2. Following the treatment, the extracts produced a dose-dependent significant reduction ($p < 0.05$) in blood glucose levels of fasted normal rats. A remarkable decrease in the blood glucose levels was observed with doses of 400 mg/kg compared to 200 mg/kg in the three plant extracts. The induced significant dose-dependent reduction in blood glucose of hyperglycemic rats ($P < 0.05$ to 0.003) could occur partly by stimulating insulin production from the pancreatic islets, or they could stimulate insulin production and glucose utilization similar to glibenclamide due to the presence of certain hypoglycemic bioactive components in their ethanolic extracts.

The three plant extracts have shown moderate brine shrimp lethality and the LC_{50} values were found to be higher than that of the standard drug. Maximum mortalities took place at the concentration 1000 µg/ml, whereas least mortalities took place at the concentration 10 µg/ml (Table 3). The two plants, sage and eucalyptus, are well known plants and their variable biological activities and chemical constituents have been extensively investigated. Antioxidant and antidiabetic effects

Table 1. Phytochemical screening of ethanolic leaf extracts of tested plants on hyperglycemic rats.

Metabolites	*E. globulus*	*S. officinalis*	*G. senegalensis*
Alkaloids	±	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Cardiac glycosides	-	-	-
Saponins	+	+	+
Anthracenes	-	-	-
Coumarins	-	-	-
Carbohydrates	+	+	+
Reducing Sugars	-	-	-
Sterols and triterpenes	+	+	+

+, Positive result; -, negative result; ±, trace amount of metabolites.

Table 2. The effect of ethanolic leaf extracts of tested plants on hyperglycemic rats*.

Group dose in mg/kg		Hypoglycemic effect of extracts after glucose loading			Reduction of glucose level within (%)	
		0 h	2 h	4 h	2 h	4 h
NCG	10	104.7767 ±15.3648	129.4250 ± 7.6297	130.2830 ± 3.9234	23.52	24.26
PCG	10	83.5966 ±9.9651	73.4433 ±9.7994	77.3650 ± 23.4247	12.15	7.45
I	200	100.7383 ±11.3558	95.3923 ±8.9228	89.0475 ± 10.8479	5.30	11.60
	400	94.7673 ±1.6962	81.9566 ± 22.7492	89.4048 ± 4.0497	29.49	23.08
II	200	111.7967 ±21.5753	87.0316 ± 27.1992	93.9501 ± 16.8295	22.15	15.96
	400	116.1617 ±21.0792	85.1133 ±8.5638	76.9266 ± 11.0213	26.72	33.77
III	200	88.0000 ± 11.0000	113.0000 ±13.0000	101.0000 ± 8.0000	28.40	14.77
	400	83.0000 ±3.0000	120.001 ±5.0000	99.0000 ± 6.000	44.57	19.27

NCG, Negative control group (distilled water); PCG, positive control group (Glibenclamide); I. Ethanolic leaf extract of *E. globulus*; II. Ethanolic leaf extract of *S. officinalis*; III. Ethanolic leaf extract of *G. senegalensis*. * Data are expressed in mean ± standard deviation significant at P-value < 0.05.

Table 3. Brine shrimp lethality assay of ethanolic leaf extracts of tested plants on hyperglycemic rats*.

96% ethanolic extract	Dose (µg/ml)	No of tested shrimps	No of survivors	LC_{50} (µg/ml)
E. globulus	10	30	30	55.954
	100	30	30	
	1000	30	29	
S. officinalis	10	30	30	37.208
	100	30	28	
	1000	30	21	
G. senegalensis	10	30	29	26.940
	100	30	29	
	1000	30	27	

* LC_{50} of the standard drug etoposide was 7.462.

are among the medicinal properties attributed to both plants. The effects of their aqueous and ethanolic extracts on serum glucose and insulin in diabetic rats and on lipid peroxidation and selected enzymes of rat liver along with their common uses in traditional medicine were reported in the current literature (Akolade et al., 2012; Arise et al., 2009; Donga et al., 2011; Eidi et al., 2005; Mishra et al., 2009). However, these activities still lack of biological experimental confirmation. It has also been established that the composition pattern of essential oils and other secondary metabolites are affected by factors such as geographical location and seasonal variations, which consequently influence their biological activities. It is on this basis that we investigated the effect of leaf ethanolic extracts of two Algerian plants grown in northern Africa on diabetic rats and compared results with a third plant grown in central Sudan with similar uses and chemical composition. The scant data on the chemical composition and experimental confirmation on the claimed hypoglycemic properties of *G. senegalensis*, gave solid grounds to our findings as a new addition to the current literature.

The resemblance in phyto-constituents and anti-diabetic effect of the three African plants grown in different geographic locations could lead to more studies to prepare herbal mixtures of the three plants bearing in mind their low toxicity and wide distribution.

In conclusion, the results of the present experimental animal study clearly demonstrated that the ethanolic leaf extracts of *E. globulus*, *S. officinalis* and *G. senegalensis* possessed a remarkable blood-glucose lowering potential in glucose loaded rats with minimum toxicity in the brine shrimp assays.

ACKNOWLEDGEMENTS

The authors are grateful to the UMST administration and Faculty of Pharmacy for help and financial support. Part of this work was carried out at MAPRI, NCR (Sudan), which is gratefully acknowledged.

REFERENCES

Akah PA, Okafor CL (1995). Blood Sugar Lowering of *Vernonia amygdalina* (Del) in an experimental rabbit model. Phytother. Res., 6: 171-173.

Akolade JO, Olajide OO, Afolayan MO, Akande SA, Idou DI, Orishadipe AT (2012). Chemical composition,antioxidant and cytotoxic effects of Eucalyptus globulus grown in north-central Nigeria. J. Nat. Plant Resour., 2(1): 1-8.

Alireza N, Mohammad B (2009). Attenuation of Oxidative Stress in Streptozotocin – induced diabetic rats by *Eucalyptus globulus*. Indian J. Clin. Blochem., 24(4): 419-425.

Alison MG, Peter RF (1998). Antihyperglycemic action of *Eucalyptus globulus* are associated with pancreatic and extra- pancreatic effects in mice. J. Nutr., 128: 2319-2323.

Alves TMA, Silva AF, Brandao M, Grandi TSM, Smania A, Zani CL (2000). Biological Screening of Brazilian medicinal plants. Mem Inst Oswaldo Cruz, 95: 367-373.

Arise RO, Malomo SO, Ackbayo JO, Igunnu A (2009). Effects of aqueous extract of Eucalyptus globulus on lipid peroxidation and selected enzymes of rat liver. JMPR, 3(2): 77-81.

Azza OF, Afaf IA, Mohamed GM (2009). Toxicopathological effects of *Guiera senegalensis* extracts in wistar albino rats. J. Med. Plants Res., 3(10): 699-702.

Borries RP (1996). Natural Product researches, Perespective from a major pharmaceutical company. Ethnopharmacology, 51: 29-38.

Caballo LJ, Hernandez LZ, Perez P, Gravalos MD (2002). A comparison between two brine shrimp assays to detect *in vitro* Cytotoxicity in marine natural products. Biomed .Cent. Biotechol., 2: 1-10.

Cristova FL, Marisa FA, Rita A, Manuel FF, Cristina PW (2006). Metformin – like effect of *Salvia officinalis* (Common sage). Br. J. Nutr., 96: 326-333.

Donga JJ, Surani VS, Sailor GU, Chauhan SP, Seth AK (2011). A systematic review on natural medicine used for therapy of Diabetes mellitus of some Indian medicinal plants. Pharma. Sci. Monitor, 2(1): 36-72.

Eidi M, Eidi A, Zamanizadeh H (2005) Effect of Salvia officinalis L. leaves on serum glucose and insulin in healthy and streptozotocin-induced diabetic rats. J. Ethnopharmacol., 100: 310-313.

Gamal EBE, Mahgoub SE, Awatif ABE, Wail SA, Mohamed GM (1998). Medicinal Plants of Sudan Part IV. Omdurman Islamic University printing and publishing House, Khartoum, pp. 73-74.

Kivack BMT, Tansel H (2001). Antimicrobial and Cytotoxic activities of *Ceratonia siliqua* L. extracts. Turk. J. Biol., 26: 197-200.

Konuklugil B, Deniz G, Yilid O, Senoz S (1997). Hypoglycemic effect of *Teucrium nucifera* extract. Phytother. Res., 5: 217-223.

Mayer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nicholas PE, Mclaughin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica, 45: 31-34.

Mishra SB, Rao ChV, Ojha SK, Vijayakumara M, Verma A (2009). An analytical review of plants for anti-diabetic activity with their phytoconstituent and mechanism of action. IJPSR, 1(1): 29-46.

Silva O, Barbosa S, Diniz A, Valdeira ML, Gomes EA (1997). Antiviral activity of *Guiera senegalensis* plant extracts against Herpes simplex virus types 1 and African Swine fever virus. Int. J. Pharm., 12: 323-325.

Saadabi AMA, Ayoub SMH (2009). Comparative bioactivity of *Hydnora abyssinica* A Braun against different groups of fungi and bacteria. J. Med. Plants Res., 3(4): 262-265.