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Hypoglycaemic Activity of *Centella Asiatica* (L) Urb

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The water extract of whole plant of *Centella asiatica* is used by traditional healers in the Haya tribe in Bukoba Region in Tanzania, in the management of both insulin and non-insulin dependent diabetes mellitus. *Centella asiatica* administered orally at a dose of 2 g/kg and 4 g/kg body weight produced a significant hypoglycaemic activity (P <0.05) in glucose primed fasted rabbits, with an average % mean deviation of 25.6 and 34.9 respectively, at a dose dependency ratio of 2:3. Fractions containing quaternary amine and triterpene given at a dose of 400 mg/kg body weight in fasted glucose primed rabbits produced a significant hypoglycaemic effect with a mean deviation of 74 % (quaternary amine) and 84% (triterpene) respectively, compared to tolbutamide that produced 62 % mean deviation. Unlike tolbutamide, *Centella asiatica* did not reduce blood sugar below normal levels. The aqueous extract of *C. asiatica* also significantly enhanced the uptake of glucose into isolated rat hemidiaphragm, incubated at 37 ^{0} C for 3 h in Glucose Kreb Ringers buffer (GKBR) solution. Glucose uptake induced by *C. asiatica* extracts was comparable with the absorption caused by insulin. Glucose uptake was most significant in leaves, followed by roots, whole plant and stems. The experiment confirms the rationale of the use of *Centella asiatica* in both type 1 and type 11 diabetes mellitus.

Key Words: *Centella asiatica*, hypoglycaemic effect, rabbits, rat hemidiaphragm.

INTRODUCTION

Centella asiatica (L) Urbana (Umbeliferaceae) is a liana well distributed in the tropics. In Tanzania it thrives well in high altitude areas with moderately low temperatures and high rainfall [1]. It is found in the north-eastern mountains of Lushoto and Muheza, southern highlands in Mbeya, and north-western areas along the eastern coast of Lake Victoria. *C. asiatica* is known as "Kutwikumoi" and "Butikwa" among the Haya. The names can be translated as single eared, which describes the leaf morphology, resembling a human ear, with a long stalk and its attribute as hunger inducing agent, respectively. It is from this use that we decided to investigate its hypoglycaemic effect.

The water extract of the whole plant is used by traditional healers in Bukoba district in Kagera region for the management of both insulin dependent (IDDM) and non-insulin dependent (NIDDM) diabetes mellitus. The plant is also used to induce appetite in post puerperal mothers and in the treatment of flatulence in newly born babies. Flesh leaves of *C. asiatica* are mixed with spinach and given to breast feeding mothers as lactagogue. Water extract made from a mixture of *C. asiatica, Crassocephalum vitellinii, Ocimum suave* and *Combretum zeylanicum* is given for malaria and flu [2]. *C. asiatica* has also been found to improve the power of concentration, general mental ability and behaviour of mentally retarded people. It possesses brain-invigorating effect and was found to increase intelligence quotient (IQ) [3]. *C. asiatica* is used in India in the treatment of rheumatism, and in increasing memory, CNS stimulant and antispasmolytic effect [4]. The crude extract of the whole plant has tested positive for antifertility effect, endothelial system stimulating and immunomodulating effects [5]. The analgesic effect of *C. asiatica* has been utilised in the management of toothache by placing heated fresh leaves directly on the tooth [6]. A decoction of very young shoots is given for haemorrhoids. It has also been valued as a tonic

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in bronchitis, asthma, gastric catarrh, leucorrhoea, kidney troubles, urethritis and dropsy [7]. A syrup of the leaves mixed with ginger and black pepper is taken for cough while leaf juice with palm jaggery is given to women as a tonic after delivery in India. The leaf juice is rubbed on the forehead to cure severe headaches [8]. Asiaticoside is useful in the treatment of leprosy and certain types of tuberculosis. This active principle dissolves the waxy covering of *Bacillus leprae*, so that the causal organism becomes very fragile and maybe easily destroyed by specific treatments [9]. Amino acid study of the plant indicated that leaves, petioles and stolons contain glutamate and serine while roots are rich in aspartate, glutamate, serine, threonine, alanine, lysine, histidine and amino butyrate [10].

The triterpenoid fraction has shown anti-inflammatory effect, healing of wounds and abscesses. The anti-inflammatory effect of *C. asiatica* was found to be associated with the inhibition of glucuronidase aryl sulphatase and glucuronic acid by madecasol, madecassic acid and asiaticoside that stabilises glucuronic acid, a major cell binding agent in humans. These compounds are also responsible for the acceleration of cicatrisation, grafting of wounds and healing of gastric ulcers [11]. Triterpene glycosides have been used to make endurance mixtures and in the management of fainting and unconsciousness. It is also used as a CNS stimulant and tonic in the treatment of epileptic seizures [12].

Water extract from the whole plant of *C. asiatica* has shown cardiotonic, neuroerectic and hypotensive activities. Glycosides in acetone extract of aerial parts have shown angiotensin-converting enzyme inhibition and cholecystokinin binding effect [13]. *C. asiatica* ethanol extract inhibited metrazole and strychnine induced convulsions and has shown anti-anxiety effect measured by blocking dopamine and serotonin receptors.

Several compounds have been isolated from *Centella asiatica* including the flavanoids kaemferol-7-O- ß-d-glycoside and qercetine-3-ß -d-glucose; and the triterpenes adecassoside, madecasic acid, and madasiatic acid, asiaticoside B, centellic acid, centoic acid, centelose glycoside, thankunic acid and isothankuniunic acid. The glycosides, brahmoside and brahminoside with their genin brahmic acid, isobrahmic acid and betulic acid have been isolated [14, 15].

The aim of the present study was to investigate the hypoglycaemia induced by *C. asiatica* extracts in normoglycaemic and glucose primed animals. In addition, glucose uptake by rat hemidiaphragm induced by *C. asiatica* extracts was compared to that induced by insulin using tissues from several animals.

EXPERIMENTAL

Reagents

Glacial acetic acid, benzoic acid, o-toluidine, thiourea, trichloroacetic acid, methanol, ethanol, chloroform, n-butanol, petroleum ether (40^o-60^o C), activated charcoal, aluminium oxide (neutral grade), D (+) glucose, and silicagel GF 60 were of analytical grade and were obtained from Merck (Darmstadt, Germany) or BDH chemical Ltd (Poole, England). Tolbutamide was obtained from Langarp Pharmaceutical Ltd (Bordohants, England). Insulin lente was obtained from Abbott Laboratories. Blood glucose was estimated with Dextrostix® strips using a reflectance meter (Ames Co, Stocks Page UK), checked with the o-toluidine method. All glassware in contact with the solutions was thoroughly cleaned with chromic acid and distilled water. Double distilled water was used for all experiments. Krebs-Ringer's buffer solution (KRB) was made by dissolving; Sodium chloride 6.9 g, 3.5 ml of 10% potassium chloride, 2.9 ml of 10 % magnesium sulphate, 2.1 g sodium bicarbonate, and 2.5 g calcium chloride in 1000 ml distilled water. Glucose was dissolved in KRB at a concentration of 300 mg/ml to get the incubation media Glucose Krebs-Ringers Buffer solution (GKRB) [16].

Plant Collection and Identification

C. asiatica was first collected from Kagera region in 1988 and it was deposited at the Institute of Traditional Medicine (ITM) herbarium (TMRU number 4693) and identified by Mr E.B. Muhoro. Mr. Mbago did the authentication at the Botany Department, Faculty of Science, University of Dar es Salaam. Further collections were from Lushoto district in Tanga region and Tukuyu in Mbeya region.

Plant Preparation and Extraction

The plant samples were dried in shade for 10 days. The dried material (1.2 kg) was ground using a harmermill and sieved through a 1 mm

sieve. The ground material was extracted by percolation first with petroleum ether (40^0-$60\ ^0$C) for 24 h at room temperature, and then with 40 % ethanol. The ethanol extract was dried on a rotary evaporator *in vacuo* at temperature not exceeding 40 ^{o} C. Final drying was done on a freeze drier, thus obtaining 200.0 g (16.7 %) of a sweet smelling dark brown powder (extract A). Extract A (50.2 g) was triturated with minimum distilled water (40 ml) and then fractionated with butanol. The butanol extract was concentrated and dried *in vacuo* to yield 28.5 g of the material which was refluxed with ethanol and shaken on an electric shaker for 2 hrs, filtered and separated into ethanol soluble, (Extract B, 4.2 g) and ethanol insoluble, (Extract C, 13.4 g). Extract C (10.3 g) was dissolved with 20 ml distilled water and the solution mixed with 30.0 g aluminium oxide. The mixture was dried *in vacuo* and applied on a column containing 150 g of aluminium oxide, packed using chloroform: ethanol: water (10:7:3 v/v). The column was eluted using the same solvent. The spots were monitored on TLC aluminium coated plates using Dragendorf reagent spray [17]. Dragendorf positive elusions were mixed, the solvent removed and the residue crystallised from ethanol, yielding 4.5 g of a fraction containing quaternary amines. Extract B was concentrated and dried *in vacuo* to yield brownish sticky oil. On addition of acetone 8.1 g of yellow amorphous precipitates containing triterpenes were obtained. The triterpenes were identified as purple spots on vanillin-sulphuric acid reagent spray on a TLC silica gel plates developed in chloroform: methanol: water (64: 50:10 %v/v) and heating at 110 ^{0}C for 5 min [17]. The leaves (0.5 kg), roots (0.5 kg), stems (0.5 kg) and 1.0 kg whole plant were then extracted separately by percolation for 24 h with distilled water. Acetic acid 1% was added to prevent the action of bacteria. In addition, similar amounts of roots, leaves, stems and the whole plant were extracted by percolation with absolute ethanol for 24 hrs. In both cases the marc was squeezed to remove the extract as much as possible. The ethanol extract was evaporated to dryness *in vacuo* at temperature not exceeding 40 ^{0}C the aqueous extract was concentrated on the rotary evaporator and then freeze dried. Dry extracts were kept tightly closed in amber colour glass containers and stored at -20 ^{0}C until use.

Hypoglycaemic Activity in Rabbits

White albino Adult male and female rabbits weighing between 1000-3000 g were purchased from a farmer and kept in the animal house at Muhimbili University College of Health Sciences. They were maintained at room temperature for 72 h, given a balanced diet of growers mash and water *ad libitum*. The rabbits were starved for 20 h, and then divided into two groups: Group A was subjected to experimentation without prior glucose load. Group B was glucose primed using 140 mg/kg body weight. Each group was then subdivided into six subgroups of six animals (A1-A6 and B1-B6). Groups A1 and B1 were administered with distilled water, groups A2 and B2 were treated with crude extract A at a dose of 2 g/kg body weight, while group A3 and B3 were treated with crude extract A at a dose of 4 g/kg-body weight. Groups A4 and B4 were treated with quatenary amine fraction (400 mg/kg body weight) and groups A5 and B5 were given the triterpene fraction (400 mg/kg body weight) and groups A6 and B6 were treated with tolbutamide 400 mg/kg body weight. The rabbits were restrained by hand and the ears were cleaned with xylene, to remove hairy fats. The blood was drained from the marginal ear vein using a 2 ml blood pipette and kept in test tubes containing sodium citrate as an anticoagulant. Blood was collected before administration of glucose and then after glucose administration, at times 0, 30, 60, 90 and 120 min.

Blood glucose was estimated with Dextrostix ® strips using a reflectance meter checked with the o-toluidine method. The percentage mean of glucose deviation at time (t) minutes was calculated from the regression line of change in concentrations with time based on the formula.

$$\%\ \ mean\ deviation\ \ = \left(\frac{G_x - G_o}{G_o}\right).100 \qquad (1)$$

$$G_o = Initial\ \ \ glucose\ level$$

$$Gx = Induced\ \ \ glucose\ level$$

RESULTS AND DISUSSION

Hypoglycaemic Activity in Rabbits

Table 1 shows the effect of *C. asiatica* extracts on the blood glucose levels of normoglycaemic animals. The water extracts (2 g/kg and 4g/kg) and the quartenary amine extract showed no significant reduction of blood glucose levels throughout the study time.

The triterpene extract had a significant effect on blood glucose, with initial glucose deviation of more than 30% and final deviation of more than 50%, similarly to tolbutamide. Table 2 shows the effect of the *C. asiatica* extract on the blood glucose in glucose primed animals. Animals treated with distilled water did not show any significant blood glucose deviation (P>0.05). The water extract (2g/kg had little effect on the blood glucose while the higher dose of water extracts (4 mg/kg) caused a mean deviation of more than 70 % at 120 mins. The quaternary amine extract behaved very much like tolbutamide, with a mean glucose deviation of about 60% at 30 min and more than 70% at 120 min.

The triterpene extract was more active than tolbutamide both at 30 min and 120 min, with a final glucose deviation of 94.4%.

The hypoglycaemic effect of *C. asiatica* crude extracts is dose related as it is higher with 4 mg/kg body weight as compared to 2 mg/kg body weight. A triterpene fraction caused higher hypoglycaemia in glucose primed animals compared to tolbutamide throughout the study period. Quaternary amine fraction also possesses hypoglycaemic effect similar or slightly milder compared to triterpene and tolbutamide. The presence of two or more active compounds may produce a synergistic effect in the total extract.

Table 1: Effects of *C. asiatica* on blood glucose of normoglycaemic animals

		Sampling Time (Min)				
Treatment	**Parameter**	**0 Min**	**30 Min**	**60 Min**	**90 Min**	**120 Min**
Vehicle treated (control)	Blood glucose	4.4 ±0.12	3.7±0.30	4.4 ± 0.6	3.3±0.93	3.0 ±0.3
	% Mean dev.		7.4±+0.24	16.3 ± 1.2	21.7±1.4	28.8±0.3
	p		P >0.05	P >0.05	P >0.05	P >0.05
Water extract 2 g/kg	Blood glucose	4.4+0.12	4.0± 0.75	3.70 ± 0.9	3.4±0.8	3.0±0.8
	% Mean dev		9.9± 0.75	24.2 ± 1.8	30.4±3.4	38.1±1.6
	p		P >0.05	P >0.05	P >0.05	P >0.05
Water extract 4 g/kg	Blood glucose	4. 3+0.2	3.9± 0.8	2.7 ± 0.3	2.6±0.1	2.3±0.2
	% Mean dev.		12.8±1.9	33.4 ± 2.4	38.6±4.4	51.1±3.6
	p		P >0.05	P >0.05	P >0.05	P >0.05
Quartenary amine 400 mg/kg	Blood glucose	4.4±0.5	3.9±0.3	2.8 ± 0.2	2.4±0.2	2.1±0.2
	% Mean dev.		9.9±0.75	32.5 ± 2.4	45.6±2.7	51.1±3.6
	p		P >0.05	P >0.05	P >0.05	P >0.05
Triterpene 400 mg/kg	Blood glucose	4.6±0.4	2.82 ± 0.02	2.6 ± 0.3	2.3±0.2	2.1±0.1
	% Mean dev.		34.5 ± 1.9	43.5 ± 4.3	50.1±4.5	54.3±3.6
	p		P < 0.05	P < 0.05	P < 0.05	P < 0.05
Tolbutamide 400 mg/kg	Blood glucose	4.5±0.1	3.0 ± 0.1	2.8 ± 0.2	2.6±0.1	2.0±0.1
	% Mean dev.		32.6 ± 2.9	37.8 ± 3.2	42.2±1.8	56.5±2.3
	p		P < 0.01	P < 0.01	P < 0.05	P < 0.05

Blood glucose values are in mmols/ml n = 6 n: number of animals, P: significant difference compared to zero, P>0.05: non significant difference compared to zero, P< 0.05: significant difference compared to zero, P< 0.01: highly significant difference compared to zero

Table 2: Effects of *Centella asiatica* on the blood glucose of glucose primed animals

Treatment	Parameter	SAMPLING TIME (MIN)				
		0 Min	30 Min	60 Min	90 Min	120 Min
Vehicle treated control	Blood glucose	9.3±1	7.7±0.2	7.1±0.1	6.4±0.3	4.5±0.2
	% Mean dev.		17.2±0.6	23.9±3.0	20.9±3.5	45.1±0.8
	p		P >0.05	P >0.05	P >0.05	P >0.05
Water extract 2 g/kg	Blood glucose	8.0±0.2	5.3±0.2	4.9±0.2	4.5±0.2	3.8±0.2
	% Mean dev.		29.8±3.3	35.4±1.0	35.8±1.6	54.5±0.9
	p		P< 0.05	P < 0.05	P< 0.05	P<0.05
Water extract 4 g/kg	Blood glucose	8.3±0.7	4.4±0.3	3.9±0.2	3.7±0.2	2.9±0.2
	% Mean dev.		47.0±3.1	63.9±3.1	66.3±2.9	71.3±7.8
	p		P< 0.05	P< 0.05	P< 0.05	P<.05
Quartenary amine 400 mg/kg	Blood glucose	8.3±0.6	3.2±0.2	3.0±0.1	2.8±0.1	2.6±0.1
	% Mean dev.		61.5±3.1	65.9±3.1	66.3±2.9	71.3±7.8
	p		P< 0.05	P< 0.01	P< 0.05	P< 0.01
Triterpene 400 mg/kg	Blood glucose	8.5±0.5	2.4±0.1	1.9±0.2	1.3±0.1	0.8±0.1
	% Mean dev.		71.8± 3.6	77.6±1.9	83.8±2.5	94.4±3.1
	p		P<0.01	P< 0.01	P< 0.01	P< 0.01
	Blood glucose	8.2±0.4	3.1±0.4	2.9±0.2	2.4±0.2	2.0±0.1
	% Mean dev.		62.2±4.3	64.6±3.5	68.9±2.7	74.7±3.1
Tolbutamide 400 mg/kg	p		P< 0.01	P< 0.01	P< 0.01	P< 0.01

Blood glucose values are in mmols/ml n = 6 n: number of animals, P: significant difference compared to zero, P>0.05: non significant difference compared to zero, P< 0.05: significant difference compared to zero, P< 0.01: highly significant difference compared to zero

CONCLUSIONS

Having found that *C. asiatica* has anti-diabetic profile of activity similar to tolbutamide; it could replace some of the marketed oral anti-diabetic drugs if dosages are standardized. The presence of three groups of compounds which act in conjunction/additively may authenticate the advantage of using *C. asiatica* in a crude form rather than pure isolates. *C. asiatica* is an example of active preparations, which can be given in crude form for common ailments while monitoring quantitatively the presence of active compounds. Further research should be done to establish dosage and improved formulation, which can have longer shelf life.

The World Health Organization (WHO) expert committee on diabetes recommended that traditional methods of treatment for diabetes should be investigated (22). Research into botanical substitute for the existing antidiabetic agents may lead into new molecules stimulating endogenous insulin biosynthesis, secretion and promotion of insulin action or having similar mechanism of action as insulin.

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