

ANTIDIABETIC EFFECT OF *EMILIA SONCHIFORA* IN DITHIZONE DIABETIC RATS.

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ABSTRACT

The antidiabetic effect of a crude extract of *Emilia sanchifora* leaves was studied in rats with dithizone induced diabetes in rat. Three different concentrations of the crude chloroform – methanol extract were given orally to both normal and diabetic rats. The percentage blood glucose reduction for ES1 was 33.3, 14.2, 45.9, 49.7, 32.9, 31.1 and 17.3% after 0, 6, 12, 18, 24, 30 and 36 hours of oral administration. Significant difference of ($P < 0.001$) occurs when the hyperglycaemic level of ES1 was compared with each group showing a remarkable reduction in blood glucose. The difference was also significant at $P < 0.01$ with normal group and after six hours of oral injection of the extract. This shows that dithizone was able to induce significant ($P < 0.01$) increase in blood glucose level. The extract showed a concentration dependent effect on blood glucose reduction hence ES3 (1.2 mg/ml) extract significantly ($P < 0.001$) reduced blood glucose than ES1 (0.4mg/ml) after 12, 24 and 30 hours of oral injection when compared to ES2 (0.8 mg/ml) extract, the reduction in blood glucose was significant ($P < 0.001$) after 6, 12, 18, 24 and 36 hours of oral administration. Chlorpropamide (DA) (diabinese) a known antidiabetic drug had the same glucose reduction pattern with the extracts. The peak percentage glucose reduction for DA, ES1, ES2 and ES3 were 32.3 after 24hours, 49.7 after 18 hours, 37.5 after 12 hours and 61.1% after 18 hours respectively. ES3 had the highest percentage reduction though DA has longer time of action. The CO group showed raised levels of blood glucose throughout the work and showed significant difference $P < 0.05$ with ES1 after 6, 12, 24 30 and 36 hours of oral injection

KEY WORDS: Dithizone diabetes, *Emilia sanchifora*, Chlorpropamide and Diabetes mellitus

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders that result in hyperglycemia due to decreased insulin production or inefficient insulin utilization. Approximately 16 million people in the United States currently have diabetes (American Diabetes Association, 2002). Prevalence of diabetes in some countries has reached up to 1 – 2% of the population and Barnett (1991) showed that in Africa it is on the increase: Ike (2001) observed that 140 million people worldwide and 1 million Nigerians are suffering from diabetes. The World Health Organization predicted that the number of diabetic patients will double from 143 million in 1997 to about 300 million in 2025 largely because of dietary and other lifestyle factors (Seicell, 2000).

The incidence of type II diabetes is closely linked to choice of diet leading to overweight or obesity (Wannamethee and Shaper, 1999). About 75% of diabetes is type II or non – insulin dependent diabetes (NIDD) (Barnett, 1991) and is associated with other disease conditions like obesity; (Wolf and Colditz, 1998); coronary heart, eye, renal, vascular and neurological problems (Miller, 1991). The use of most synthetic antidiabetic drugs like

sulfonylurea, biguanides and intravenous insulin injections have their own disadvantages. The most important side – effect of sulfonylureas is hypoglycaemia (Berger, 1985). The severe hypoglycaemia can lead to death.

Insulin injection takes place intravenously which is painstaking and does not improve type II diabetes (McCarty, 1998). Insulin is also frequently destroyed in the gastrointestinal tract. Insulin degradation and presence of insulinase were reported by many authors (Kitabchi and Stentz, 1972; Kahn, et al 1976). There is therefore need for oral substitutes for both insulin and severe hypoglycaemic antidiabetic drugs in management of diabetes.

In folk medicine many leaves are allegedly used for the treatment of diabetes. Prominent among these is *Emilia sanchifora* whose leaves are mashed, extracted and taken orally. Many other plants and their extracts have been used as oral substitutes. These include *Trigonella foenum gracum* (Fetrow and Avila, 1999) *Pterocarpus marsupium* (Maries and Farnsworth 1995) *Vigna unquiculata* (Tella and ojihomon, 1980) and *Aloe barbadensis* (Yongchiayudha et al, 1996).

TABLE 1.0: EFFECT OF CM EXTRACT OF EMILIA SANCHIFORA ON BLOOD GLUCOSE LEVELS

SAMPLE	NORMAL	HYPERGLYCEMIC LEVEL	TIME - (HOURS)						
			6	12	18	24	30	36	
ES1 (mg/dl)	96.01 ± 2.11 ^{bd}	145.30 ± 2.30 ^{agk}	125.33 ± 3.16 ^{bugk}	80.99 ± 5.01 ^{depl}	73.40 ± 4.65 ^{eeq}	97.30 ± 3.56 ^{depl}	100.6 ± 4.5 ^{depl}	120.35 ± 2.15 ^{degk}	
Percentage Reduction (%)	33.3	100	14.2	45.9	49.7	32.9	31.1	17.3	
ES2 (mg/dl)	96.30 ± 1.19	149.20 ± 3.6 ^{depl}	99.01 ± 3.41 ^{agel}	92.5 ± 2.91 ^{depl}	112.01 ± 2.63 ^{eeel}	101.01 ± 2.90 ^{edl}	115.60 ± 0.11 ^{edl}	119.5 ± 0.66 ^{cdgl}	
Percentage Reduction (%)	35.6	100	33.6	37.5	24.9	32.3	22.9	20.1	
ES3 (mg/dl)	96.40 ± 0.11 ^{bd}	145.16 ± 2.61 ⁱ	112.35 ± 1.91 ^{el}	87.02 ± 2.91 ^{el}	71.10 ± 3.75 ^{ee}	88.05 ± 2.58 ^{ad}	102.03 ± 2.45 ^{edl}	125.09 ± 1.58 ^{bdk}	
Percentage Reduction (%)	33.8	100	22.8	39.9	61.1	39.4	29.7	14.2	
DA (mg/dl)	98.50 ± 0.66 ^b	140.36 ± 3.6 ⁱ	124.90 ± 3.2 ^{ai}	102.45 ± 2.16 ^{el}	97.44 ± 1.16 ^e	94.0 ± 3.2 ^{el}	102.60 ± 2.31 ^{el}	106.01 ± 2.55 ^{el}	
Percentage Reduction (%)	29.9	100	21.5	27.2	30.8	32.3	27.2	24.3	
CO (mg/dl)	93.45 ± 3.45 ^b	140.06 ± 2.15 ^a	140.00 ± 1.15 ^a	142.24 ± 1.16 ^a	143.25 ± 0.95 ^a	139.04 ± 1.11 ^a	138.81 ± 1.45 ^a	141.16 ± 2.11	
Percentage Reduction (%)	28.1	100 ^c	0.01	0.02	0.03	0.01	0.02	0.01	

Values represent mean ± SEM N=3

Letters represent the Value of t when

a - hyperglycemic levels is compared with all samples at P<0.05

b - hyperglycemic levels is compared with all samples at P<0.01

c - hyperglycemic levels is compared with all samples at P<0.001

d - CO is compared with each sample at P<0.05

e - CO is compared with each sample at P<0.01

f - CO is compared with each samples at P<0.001

g - ES 3 is compared with ES2 and ES1 at P<0.05

h - ES 3 is compared with ES2 and ES1 at P<0.01

i - ES 3 is compared with ES2 AND ES1 at p<0.001

j - Normal is compared with each sample at P<0.05

k - Normal is compared with each samples at P<0.01

l - Normal is compared with each samples at P<0.001

Emilia sanchifora apart from being utilized locally has not been reported for any antidiabetic action in the literature. Hence we deem it necessary to investigate the possibility of this plant extract use to reduce blood glucose levels.

MATERIALS AND METHODS

Preparation of Extract.

The crude extract was prepared by soaking a known dry weight of the leaf in chloroform – methanol in the ratio of 2:1. The crude extract was filtered and concentrated for 24 hours. Three different concentrations ES1, ES2 and ES3 representing 0.4, 0.8 and 1.2 mg/ml of distilled water were used. Chlorpropamide (diabinese) a known antidiabetic drug was used as standard.

Treatment of Animals.

Male rats of average weight of $235g \pm 5.6$ (Mean \pm S.D), were made diabetic by injecting 50mg/kg body weight of dithizone intraperitoneally. Development of diabetes was allowed for 3 days. Another group of animals were not given the extract. This was regarded as the control group (CO). Thus 5 groups of rats were treated as ES1, ES2, ES3, DA and CO. Oral administration of 0.4mg/ml, 0.8mg/ml, 1.2mg/ml of extract and 1.2mg/ml of chlorpropamide were given orally to ES1, ES2, ES3 and DA groups respectively. Group 5, (CO), was given 1ml of normal saline. Blood glucose reduction pattern was monitored after 6,12,18,24,30 and 36hours of oral administration.

Determination of Blood Glucose Concentration

Blood glucose was measured using the method of Trinder, (1969). The glucose oxidase enzyme kit contained tris/phosphate buffer, phenol, 3,4 – dichlorophenol, fatty-alcohol-polyglycol ether, 4-aminophenazone, peroxidase and glucose oxidase. The blood was collected into a NaF bottle and separated immediately. The reagent solution (2.0ml) was mixed with 0.02ml of sample or standard provided in the kit. Incubation and absorbance were done using Reflotron (a modern discrete author analyzer (Roche) Mannheim Germany). Incubation was done at $20 - 25^{\circ}\text{C}$ for 30 minutes while absorbance was read at 510nm. Concentration of the blood glucose was calculated from the absorbance of the standard.

RESULTS.

Table 1.0 shows the glucose reduction patterns of ES1, ES2, ES3, DA and CO. Dithizone was able to induce diabetes by increasing the blood glucose levels from 96.01 ± 2.11 mg/dl of normal group of ES1 to 145.30 ± 2.30 mg/dl after 3 days.

The hyperglycaemic range was $140.06 \pm 2.15 - 149.20 \pm 3.60$ mg/dl. *Emilia sanchifora* leave extract ES1, ES2 and ES3 reduced the hypoglycaemic level significantly. Thus ES1 reduced the glucose level from 145.30 ± 2.30 mg/dl to 125.33 ± 3.16 , 80.99 ± 5.01 , 73.40 ± 4.65 , 97.30 ± 3.56 , 100.6 ± 4.5 and 120.35 ± 2.15 mg/dl after 6, 12, 18, 24, 30 and 36 hours of oral administration of extracts. Generally the reduction continued and went up to a peak. This was similarly observed in DA but not in CO. However, the level did not return again to the hyperglycaemic levels. The highest peak reduction was observed with ES3 after 18 hours of oral administration when ES1 and ES3 were compared, there was a significant concentration effect at all levels of blood collection. Thus after 6 hours ES1 reduced the blood glucose by 14.2% while ES3 was 22.8%. The same was seen after 18 hours where ES1 reduced blood glucose by 49.7% while ES3 was 61.1%. This increase in reduction of blood glucose as a result of increase in concentration of extract was significant at $P < 0.05$ at all levels of collection. The control group did not show any reduction in blood glucose levels. Dithizone induced diabetes was observed to fluctuate between hyperglycaemic levels of $138.04 \pm 1.45 - 143.25 \pm 0.95$. The chlorpropamide also reduced the blood glucose and had a longer time of action, with the peak reduction after 24 hours unlike ES3 that had its peak reduction after 18 hours. It was also observed that after 36 hours of DA oral administration, the percentage reduction was 24.3% which is higher than ES3, ES2 and ES1 of 14.2, 20.1 and 17.3 % respectively. Thus DA has longer time of action than the 3 concentrations, though ES3 has the highest percentage (61.1%) of glucose reduction.

DISCUSSION

The three different concentrations of *Emilia sanchifora* were able to reduce blood glucose levels in dithizone induced diabetes. The peak glucose reduction was observed in ES3 after 18 hours of oral administration. Chlorpropamide seems to have a longer duration of action. This is in consonance with many reports that most sulfonylureas have severe hypoglycaemic action. (Berger, 1985). Many bioactive compounds have been isolated from plants and have been used as antidiabetic agents. Examples include are *Trigonella foenum-graecum*, which is also a legume (Fetrow and Avila 1999). The mechanism of action of the hypoglycaemic effect of this plant was attributed to inhibition effects of mucilaginous fibers on glucose absorption (Sharma et.al. 1996).

The chloroform – methanol extract was comprised most of the lipid – soluble compounds in the leaf. The mechanism of blood glucose reduction of this extract may be as a result of the ability of the fat soluble extract to bind to receptor sites especially the peroxisome proliferator – activated receptors. These receptors are the chief regulators of glucose metabolism. They have the ability of binding to lipid – soluble substances because they are steroid group of receptors i.e. they are lipophilic in nature. The binding of CM extract, to this receptors may activate the receptors. Which act on glucose metabolizing pathways and thus reduce or reverse the glucose circulation in dithizone induced diabetes. Activation of these receptors also can be used to regulate gene expression, since the active form of these receptors can bind to DNA and modulates gene expression, and thus tissue – specific expression. Diabetes mellitus having a genetic origin can be attacked at this molecular level. Also since these receptors are found in almost all the tissues all other tissues diseases, associated with diabetes can also be checked. It is also interesting to note that insulin generates its intracellular effects by binding to a plasma membrane receptor, which is the same in all cells. The receptor is a disulfide – bonded glycoprotein, thus lipid – soluble CM extract may also easily bind to this receptor site at the plasma membrane, thus decreasing glucose transport by decreasing the number of glucose transport molecules in the plasma membrane (McCallium and Epanand 1995). Membrane – associated signaling processes or alterations in membrane physical properties by CM extract may be another possible mechanism of glucose reduction in this study. Many membrane bound glycolytic enzymes may be also affected, for example, insulin induces the uptake of glucose by fusion of intracellular glucose transporter – containing vesicles to the plasma membrane, this facilitates the phosphorylation of membrane phosphoinositides by activation of phosphoinositide 3 – kinase (Carpenter and Cantley, 1996) which mobilizes critical signaling enzymes and proteins.

In conclusion therefore, the chloroform-methanol extract of *Emilia sanchifora* has shown to have some antidiabetic effect in rat. The antidiabetic effect observed was concentration dependent and compared well with chlorpropamide – a known antidiabetic drug. Further work is therefore needed to detect the active antidiabetic ingredients.

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