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Evaluation of anti-hyperglycemic activity and side effects of *Erythraea centaurium* (L.) Pers. in rats

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In folk medicine, several plants, among which the small centaury, are recommended for the treatment of diabetes type 2 in humans. An experimental study to evaluate anti hyperglycemic effect of *Erythraea centaurium* (L.) Pers. was performed on wistar rats. Normoglycemic rats and rats subjected to oral glucose tolerance test overload "OGTT" were used. Administration to these animals by gavage of 20% aqueous extracts (at a dose of 0.66 ml/100 g body weight) and butanolic extract (at a dose of 0.015 ml/100 g body weight) of *E. centaurium* allowed to note, after the kinetic study of glucose between t0 and t180 min, a significant reduction in blood glucose levels. The anti hyperglycemic action of butanolic and aqueous extracts was compared to that obtained by the administration of Glibenclamide "Daonil® 5mg (ND)" at a dose of 0.25 mg/100 g weight body as a reference drug. This study showed the anti hyperglycemic property of the small centaury. But in the medium term, the administration of this plant in rats showed adverse effects on the liver and kidney.

Key words: *Erythraea centaurium*, aqueous extract, butanolic extract, anti-hyperglycemic effect, rats.

INTRODUCTION

Diabetes mellitus is one of the major diseases currently affecting the citizens of both developed and developing countries. It is estimated that 143 million people world-wide are affected by this disease and the number is growing rapidly (Maiti et al., 2004; Mentreddy et al., 2005). It is the fourth leading causes of death in the most developing countries (Arumugam et al., 2013). Some of the major reasons for the increasing rate of Type 2 diabetes also called non-insulin dependent diabetes are stress, and lack of proper diet and physical exercise (Mentreddy et al., 2005). The therapies currently available for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, a-glucosidase inhibitors, and glinides, which are used as monotherapy or in combination. Many of these oral antidiabetic agents have a number of serious adverse effects. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation (Jung et al., 2006). Plant-based medicinal products have been known since ancient times. The ethno botanical studies report about 800 plant species that may possess anti diabetic properties. Several plant species have been used for prevention or managing diabetes by the Native Americans, Chinese, South Americans and Asian Indians (Alarcon-Aguilara et al., 1998; Mentreddy et al., 2005). Traditional medicine is

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used for treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population (Saravanan and Pari, 2008). This study investigates the anti hyperglycemic activity of small centaury on rats.

The action of this herb was also compared with a sulfonyl urea, glibenclamide (Daonil ®) as a reference drug. The choice of the small centaury is based on surveys of the population and herbalists' which use this plant in some regions of Algerian country. It has been reported that the whole herb of centaury is appetite-stimulating, aromatic, bitter, cholagogic, diaphoretic, digestive, emetic, weakly febrifugal, hepatic, stomachic and tonic (Nada Mijajlović et al., 2005), hhypoglycemic, antipyretic, cardio-regulator, depurative, cicatrizing and hair-care (Bellakhdar et al., 1991). The chemical composition of the essential oil of this plant was investigated by Jerković et al. (2012).

MATERIALS AND METHODS

Plant materials

Plant identified by a specialist (Ms Khalfallah, Faculty of Science, University of Mentouri Constantine) was harvested in April in the region of Jijel and a specimen was preserved in our herbarium. The aerial parts of the plant were dried in the shade at room temperature for a period of 7 days. From the aerial parts of the plant, 4 dried extracts were obtained: dry petroleum ether, dry dichloromethane, dry ethyl acetate and 1-butanol.

Animals

Wistar adults' rats from the two sexes, with an average weight of 200 to 360 g were used. They were kept in the animal housed in cages under standard laboratory conditions and fed rabbits pellets and watered *ad libitum* until the end of the experiment.

Materials and chemicals

Balance device for restraining rats, metal cannula for feeding timer, 20 µl Eppendorf tubes containing heparin, tubes (for serum), pipettes tips (0.5A 5 ml, of 10 µl and 1 ml), bath (Grant brand temperature 20 to 90°C), glucometer (Roche) + strips, P Selecta centrifuge, precision balance (Precisa 3100C), Spatula, resistance (Stuart SB162 Series) were used for the study.

Chemicals

50% glucose solution, saline, aqueous extract, 20% butanol extract, glibenclamide (5 mg Daonil®), tranquilizers (acepromazine), anesthetics (xylocaine 2% non adrenaline) were purchased from Faculty of Science.

Experimental protocol

Rats were randomly divided in 5 groups of 4 rats each. Each rat was weighed and marked a significant sign. The day of the experiment, rats were fasted for 12 h. The different groups are the following: group 1: normal rats (NC); group 2: hyperglycemic untreated (HG); group 3: hyperglycemic treated with aqueous extract (HG + AE); group 4: hyperglycemic treated with butanol extract (HG + BE). Animals were gaved at t0 for group 1 and 2, at t60 min for groups 3 and 4, and at t5 mn for the 5th group. The blood samples were taken at t0, t60 min, t90 min, t120 and t180.

Drugs administration

Rats were overfed by plant extracts at t0 (60 min before the glucose overload); each of the animals in the (HG + AE) and (HG + BE) received the plant extract at a dose of 0.66 ml/100 g body weight and 0.015 ml/100 g body weight, respectively. Taking into account the daily amounts recommended by traditional healers for man, an aqueous extract at 20% w/v was prepared with the aerial parts of the plant (flowers, leaves and stem). The butanol extract was diluted in 1.5 ml of saline. At t60 min, each of the rats in the control group received 1 ml of absolute physiological saline (sodium chloride NaCl 9%). All other rats were given with other batches of the solution 50% glucose in distilled water (which corresponds to 0.8 ml/100 g body weight). The test was performed as described elsewhere (Alaoui et al., 1995; Hmamouchi et al., 1995). The last batch was received just before the glucose solution; the-glibenclamide was applied at standard-dose of 0.25 mg/100 g body weight (1.5 ml diluted in distilled water). The blood samples were made in caudal vein by section of the bright end of the tail, saphenous vein, after calming (acepromazine) and local anesthesia (xylocaine at 2% non adrenaline) and finally by cardiac puncture after anesthesia (just before sacrification animals).

Glycemic determination

The equipments used for the quantitative determination of glucose were the glucometer and spectrophotometer. For the 1st method, the blood glucose test-method was used directly as described by Andrade-Cetto et al. (2005) with the blood glucose meter (Accu-Chek Active, Roche Diagnostics Laboratory 2002). For the 2nd method, blood (0.5 ml) was placed in heparinized tubes and glucose was determined using the enzymatic method (glucose oxidase/peroxidase) by spectrophotometry. Three experiments were conducted:

1st experimentation

Glucose kinetics was performed on 20 rats of 5 groups; glucose was taken at t0 (basal glucose), t60 min, t90 min, t120 min and t180 min.

2nd experimentation

A blood sample taken from the saphenous vein after deep anesthesia (infiltration of 0.5cc of xylocaine 2%), skin incision and exposed surgically vessel was made at t0 for the 20 rats in the 5th groups. The glucose was performed with spectrophotometry.

3th experimentation

At the 16th day, the test of oral glucose tolerance was made alive in rats, fasted for a day. After gavage with physiological saline (for controls), serum glucose 50% (for other rats) and butanol extract to the batch. After half an hour, blood samples were performed by intra-cardiac-after stilling the acepromazine (vetranquil 1%) at a dose of 0.3 cc / animal (IM) and caudal puncture. The glycemia has been revealed by the glucometer (blood in the tail) and by enzymatic method of heparinized blood (sample intra-cardiac puncture). The animals were then sacrificed by intracardiac injection of 0.75 cc of xylocaine (lidocaine 1%).

Table 1. Effets of *Erythreae centaurium* extracts on glycemic index in normal and hyperglycemic treated rats at different time intervals.

Extract	Time (min)				
	t_0	t_{60}	t_{90}	t_{120}	t_{180}
Control	82.5±4.51	96.25±12.87	99.00±8.83	81.50±19.64	85.00±16.21
Variation (%)	100	116.67	120	103.62	103
H.G.A.E.	109.5±31.04	129.0±11.22	122.25±32.27	125.0±29.22	126.5±22.9
Variation (%)	100	118.34	112.16	114.68	116
H.G.B.E.	198.25±55.79	220.0±71.67	151.75±36.77	130.5±43.15	133.0±39.02
Variation (%)	100	110.97	76.54	65.82	65.82
H.G.D.	116.00±30.43	114.5±29.65	76.54±4.97	72.0±10.71	68.0±7.70
Variation (%)	100	98.70	72.41	62.06	58.62

H.G.A.E.: Hyperglycemic + aqueous extract; H.G.B.E.: hyperglycemic + butanolic extract; H.G.D.: hyperglycemic + daonil®.

Table 2. Effets of *Erythreae centaurium* extracts on glycemic index in hyperglycemic treated rats and control group at different time intervals.

Extract	Time (min)				
	t_0	t_{60}	t_{90}	t_{120}	t_{180}
Hyperglycemic	82.25±9.39	104.75±25.37	93.25±11.35	88.00±9.09	84.25±10.94
Variation (%)	100	122.87	109.38	103.22	98.82
H.G.A.E.	109.5±31.04	129.0±11.22	122.25±32.27	125.0±29.22	126.5±22.9
Variation (%)	100	117.80	111.64	114.15	115.52
H.G.B.E.	198.2±55.79	220.0±71.67	151.75±36.77	130.5±43.15	133.0±39.02
Variation (%)	100	110.97	76.54	65.82	65.82
H.G.D.	116±30.43	114.5±29.65	76.54±4.97	72.0±10.71	68.0±7.70
Variation (%)	100	98.70	72.41	62.06	58.62

H.G.A.E.: Hyperglycemic + aqueous extract; H.G.B.E.: hyperglycemic + butanolic extract; H.G.D.: hyperglycemic + daonil®.

Statistical analysis

The results are expressed as mean values along with their standard error of the mean (SEM). The overall statistical analysis of our results, the test of analysis of variance (ANOVA) followed by DUNNET test was used to determine statistical significance. *P* is accepted for a value equal to or less than 0.05.

Histological study

The samples were performed on different organs (liver, kidneys) for dead animals (11) and sacrificed animals (9). They were fixed in 10% formalin (37% formaldehyde) for a week and then histological investigations were performed.

RESULTS AND DISCUSSION

Glycemic evaluation

The results of the variation in glucose levels are shown in Tables 1, 2, Figures 1 and 2. The administration of *Erythrea centaurium* L. (pers.) at 0.66 ml/100 g body weight (aqueous extract), and 0.015 ml/100 g (butanolic extract) body weight and 0.25 mg/100 g body weight of Daonil® has reduced significantly glycemia compared to controls at t60, t90, t120 and t180 min. Glycemia obtained with Daonil® was significantly lower than that of (HG + BE) and (HG + AE) groups from t60 until t180 min. The difference between the (HG + AE) and (HG + BE) was most important at t0, t60 and t90 min. Data from this study showed that aqueous extract (0.66 ml/100 g body weight) and especially the butanolic extract (0.015 ml/100 g body weight) of *E*. *centaurium* L. (pers.) reduced blood glucose levels in normoglycemic and hyperglycemic rats. According to Alaoui et al. (1992), an association of three plants (*Ammi visnaga*, *Thymus ciliates* and *E. centaurium*) used in traditional medicine in Morocco has demonstrated its hypoglycemic effect in normoglycemic rats. The same plants, dried and powdered are combined in the following proportions (*A*. *visnaga* 20 g, *E*. *centaurium* 20 g and 5 g of *T*. *ciliatus*) are administered orally in the form of aqueous extract at a dose of 450 mg/kg to rats subjected to HPVO. This aqueous extract showed some anti hyperglycaemic activity against hyperglycemia induced by glucose in 30 min after administration and short-term (4 h), the aqueous decoction is significantly active. The aqueous decoction contains the association thus contains water-soluble ingredients (Alaoui et al., 1995).

Our results are also similar to those of other authors who observed that administration by gavage of 1 ml/100 g

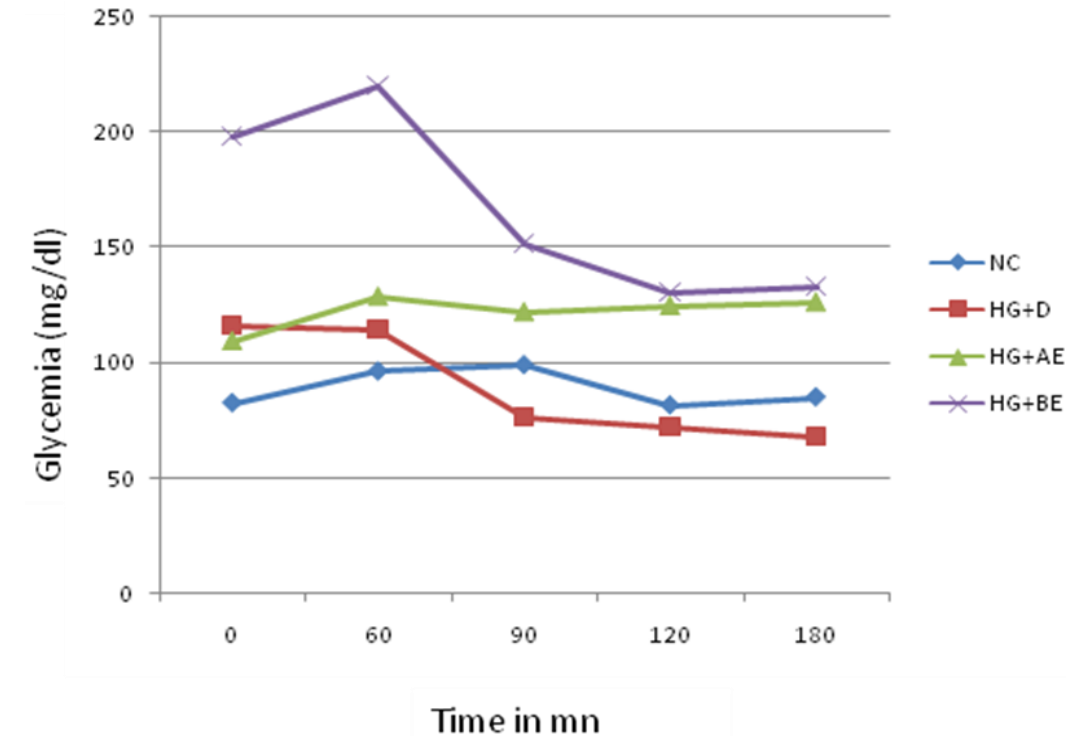

Figure 1. Glycemia of the different groups compared to normal control rats. NC: Normal control; HG + AE: hyperglycemic + aqueous extract; HG + BE: hyperglycemic + butanolic extract; HG + D: hyperglycemic + daonil®.

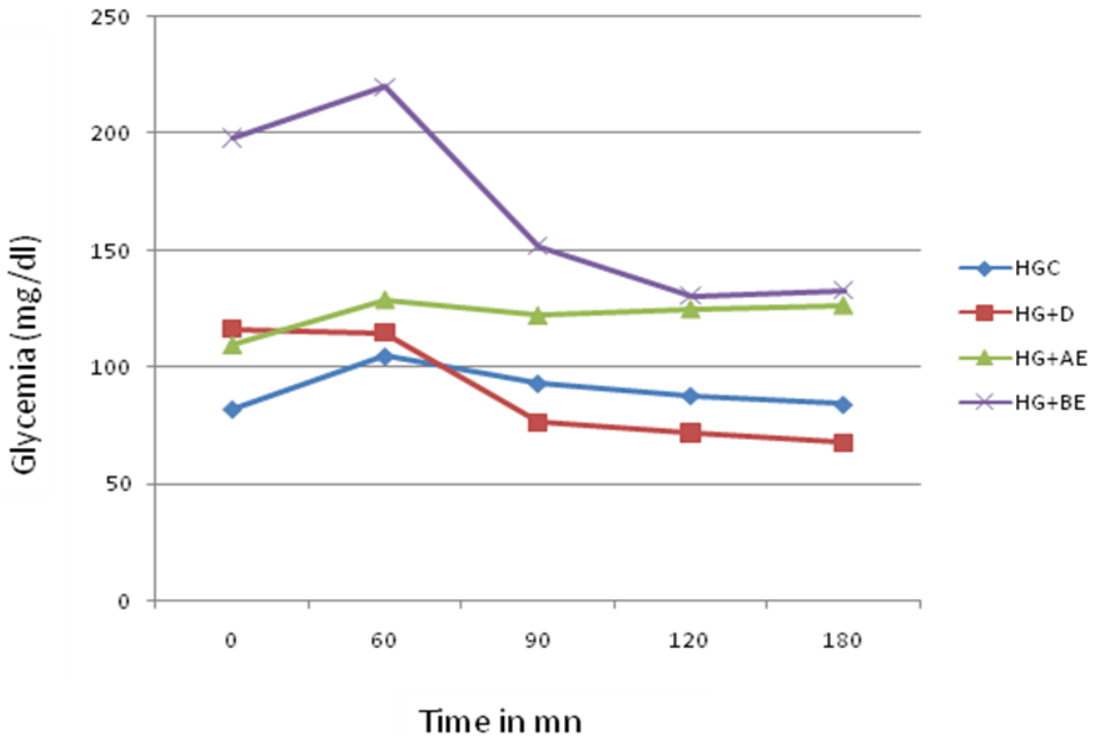

Figure 2. Glycemia of the different groups compared to the hyperglycemic control rats. HGC: Hyperglycemic control; HG + AE: hyperglycemic + aqueous extract; HG + BE: hyperglycemic + butanolic extract; HG + D: hyperglycemic + daonil®.

body weight of an aqueous decoction of 20% of each of the following plants: *Marrubium vulgare* L., *Artemisia herba-alba*, *Olea europaea* and *Zygophyllum cornutum* rats subjected to an overload of glucose HPVO revealed interesting hypoglycemic properties. The hypoglycemic effect of the extract of *Z*. *cornutum* is the most important, with a decrease in blood glucose by 46% at 60 min followed by *M*. *vulgare* L. (Hmamouchi et al., 1995). A study has evaluated hypoglycemic activity of *Zygophyllum Globularia alypum* and *gaetulum*; oral administration of these plants at a dose of 0.7 g/kg produced hypoglycemia in normoglycemic and hyperglycemic rats (Skim et al., 1999). The leaves of *Cogniauxia podoleana* also were tested for hypoglycemic activity in rats, a reduction of 21% was observed 3 h after administration by gavage to rats subjected to HPVO extracts unheated and heated administred at 250 and 500 mg/kg body weight respectively (Diatewa et al., 2000). *Anacardium occidentale* L. is a plant traditionally used in southern Cameroon to treat diabetes; the administration of the aqueous extract of the leaves of this plant, at a dose of 175 and 250 mg/kg body weight in rats after glucose tolerance test was driven attenuation of blood glucose after 1 h (Kamtchouing et al., 1998; Sokeng et al., 2001).

In a study of Sefi et al. (2011), the authors have tested the leaf extract of this plant in diabetic rats (200 mg/kg bw/day, i.p for 30 days); their study has concluded that *Centaurium erythrea* treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress and pancreatic β-cells' damage which may be attributed to its antioxidative potential. Another study of Hamza et al. (2010) conducted on diabetic male mouse induced with a standardised high fat (HFD) diet, has shown a preventive effect of *C. erythrea* extract on HFD-induced diabetes at a dose of 2 g/kg body weight daily for 20 weeks. The results of our study conducted on rats confirm the anti hyperglycemic activity of small centaury in mouse showed in the study of Hamza et al. (2010). We mention that the material plant in these two studies is from the same region (East of Algeria).

Lesions

Before the last experiment, 11 animals died within 15 days following the 1st and 2nd experiment. These animals were autopsied and histological samples of liver and kidney were made. After the 3rd experiment, all animals were killed and necropsied. The lesions found in the majority of dead animals, are congestion and hemorrhage organs (liver, kidney) and the corpse. Necrotic lesions of the liver and cystic kidneys were observed. Microscopic lesions are essentially lesions degeneration and necrosis of the liver and especially kidney degeneration (epithelium with occasional involvement of the glomerulus). Lesions congestion and hemorrhage are the signs of intoxication. It seems that the dose recommended by traditional healers is too high, at least in rat's model, which has caused the death of animals and the observation of these lesions or toxicity reported elsewhere; but for some authors, toxicity does not exist for this plant (Mroueh et al., 2004). Kidney damage due to the administration of centaury has already been reported by other authors (Haloui et al., 2000). It seems that the dose given to man by traditional healers is too high, at least in rats. We mention here a study of Tahraoui et al. (2010) conducted in rats and mice to determine potential toxicity of this plant. In acute toxicity, the authors have tested by gavage the doses of 1 to 15 g/kg (single dose in mice) and in sub-chronic studies, they have tested orally in rats 3 doses 100, 600 and 1200 mg/kg daily for 90 days. The searchers have concluded that the *E*. *centaurium* -extract is relatively non-toxic in view of their obtained results.

Conclusion

The experimental study in rats confirmed the anti-hyperglycemic effect of small centaury (*E*. *centaurium*) at a dose of 0.66 ml/100 g body weight of the aqueous extract and 0.015 ml/100 g body weight of butanolic extract and noted that the plant has side effects for a long time, at least at the tested dose. Lesions are generalized congestion, degeneration and necrosis of the liver and especially kidney. This dose, given to man by traditional healers, is too high, at least in rats. The toxicity of high-dose of this plant has already been reported by some authors.

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