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DIABETICS ENERGY SYSTEMS IMPROVED BY ANASTATICA HIEROCHUNTICA AND CLEOME CHRYSANTHA BY

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ABSTRACT

Hypoglycemic effects of Anastatica hierochuntica and Cleome chrysantha were studied in streptozotocin rats. Ether and methanolic extracts of both plants were ingested into diabetic rats to evaluate their influences on energy metabolism and cytochrome-c system in brain, liver and kidney. For energy metabolism, ATP reduced but ADP and AMP levels were elevated at control of diabetic rats. In addition, myokinase activity was stimulated at diabetic control. Ingestion of both plants ether extracts insignificantly changed diabetes effect on (ATP, ADP, AMP) myokinase. Ingestion of methanolic extract of Anastatica and Cleome elevated ATP levels but ADP and AMP were reduced. Myokinase activity was inhibited of diabetic rats. Diabetes reduced cytochrome-c level but cytochrome-c-oxidase and succinate-c-reductase activity was stimulated. Ingestion of both plants ether extracts was insignificantly changed the diabetes effects on cytochrome-c system. Both plants methanolic extracts had antagonistic effects on diabetes influences. For that, cytochrome-c respiratory system was improved nearly normal control.

Keywords: Anastatica hierochuntica, Cleome chrysantha, diabetes, ATP, ADP, AMP, myokinase, cytochrome-c-oxidase, succinate-c-reductase.

Abbreviations: ATP: Adenosine-5-triphosphate; ADP: Adenosine-5-diphosphate; AMP: Adenosine mono phosphate; STZ; streptozotocin.

INTRODUCTION

The diabetes is a heterogeneous primary of carbohydrate metabolism with multiple etiologic factors that generally involves absolute or relative insulin deficiency, insulin resistance or the both. All causes of diabetes ultimately lead to hyperglycemia, which is the half mark of this disease (Foster, 1991 and Olefsky, 1992). It is usually accompanied with glycosurea and disturbance of animal metabolism in particular the energy and cytochrome systems (Murray et al., 2006). The expenditure of energy in body is required for maintenance of ionic gradients across membranes, locomotion, synthesis of molecules, secretory processes, detoxification and heat generation. The central driving force which is the basis of all energy requiring biological processes is high energy phosphate in the form of ATP which can be generated by the oxidation of carbohydrate, fat and protein. These

sources of energy can be oxidized at different rates and in different proportions to each other, depending on the body's demand for energy and on the availability of these energy fuels to the body. The relationship between energy and cytochrome-c systems has been proved for many years (Elliott and Elliott, 2001). In diabetic animals, insulin secretion is probably backing to meet the different metabolic pathways.

Several natural medicinal agents, through of limited natural distribution have been used for the treatment of diabetes mellitus in amounts limited by tradition and personal taste. In general, no serious effects have been associated with this treatment, but it would be irrational to grant these materials exemption from modern safety testing when administrated to animals in a manner which produces any conscious control over their use by the consumer.

Anastatica hierochuntica and Cleome chrysantha which are grown wild in the Halal North Sinai are popularly known for its berrier to exhibit a variety of pharmacodynamic effects: diuretic, carminative, antiseptic and abortive among others (Gowenlock et al., 1988 and Abdel-Rahim et al., 1992). The plants ingestion into normal rats showed insignificant hypoglycemic effect in non-glycemic rats (El-Ridi, 2001 and Abdel-Rahim et al., 1992). Although several investigations were done on for treating diabetes amongst, Abdel-Rahim et al., (2005) found that Anastatica hierochuntica and Cleome chrysantha appear to have blood sugar lowering effect by improvement the carbohydrate metabolism which disturbed by diabetes in streptozotocin (STZ) diabetic rats. In the same manner, Rahmy and El-Ridi (2002) pointed out that Anastatica hierochuntica administration to STZ-diabetic rats also showed hypoglycemic effects.

The present work aims to evaluate the influence of Anastatica hierochuntica and Cleome chrysantha ingestion on energy system i.e. ATP, ADP and AMP (energy metabolites) content and myokinase activity as well as respiratory system (cytochrome-c content and the activity of cytochrome-c-oxidase and succinate-cytochrome-c-reductase) in brain, liver and kidneys tissues of STZ-diabetic mal adult albino rats.

MATERIAL AND METHODS

Anastatica hierochuntica and Cleome chrysantha as hypoglycemic agents were extracted by successive extracts using ether and methanol respectively. Each fraction was concentrated under vacuum using a rotary evaporator at 40°C and stored at -20°C until used. All the fractions were suspended at different concentration of water or cotton seed oil before oral ingestion into the diabetic rats by daily doses of 150 mg/kg body weight for 6 weeks of each plant extracts.

A total of 36 adult male albino rats of Wister strain (three months old and weight average of 150 g) were kept separately in well aerated cages under hygienic condition. Rats were maintained with free access to water and a standard diet consisting of casein 15 %, cotton seed oil 10 %, salt mixture 4 %, vitamins mixture 1 % and starch 65 % (Lane-Petter and Pearson, 1971) for two weeks (adaptation

period). Diet and water were supplied ad Libitum. To induce diabetes, 30 rats were injected with a single dose of streptozotocin (60 mg/kg body weight) given intraperitoneally (i. p.) to rats according to the method described by Adeghate et al. (2001). Blood samples were withdrawn from orbital venous plexuses and blood glucose was determined. The diabetic rats were then randomly assigned and divided into 5 groups each of 6 rats and fed on the basal diet.

The first group (6 normal rats without injection of streptozotocin) was used as normal control. The 2nd group (6 of diabetic rats) was used as diabetic control. The 3rd group (6 of diabetic rats) was ingested daily with a dose of 150mg/kg body weight of ether extract of *Anastatica hierochuntica*. The 4th group (6 of diabetic rats) was ingested with 150mg/kg body weight of methanolic extract of *Anastatica hierochuntica*. The 5th group (6 of diabetic rats) was ingested with 150mg/kg body weight of ether extract of doum (*Cleome chrysantha*). The 6th group (6 of diabetic rats) was ingested with 150mg/kg body weight of methanolic extract of *Cleome chrysantha*. All the 6 groups animal were fed on the basal diet for 6 weeks (experimental period). Then all rats were killed by decapitation. Blood, brain, liver and kidneys of the experimental animals were removed and chilled up for analysis.

Enzymatic determination of rats plasma glucose was carried out according to Trinder method (1969). Determination of adenoins-5-triphosphate (ATP), adenosine-5-diphosphate (ADP) and adenosine-5-monophosphate (AMP) contents as well as the activity of myokinase in brain, liver and kidneys tissues were done as described by Bergmeyer (1974). The organs tissue mitochondria were isolated and purified then emulsified with 1% triton x-100 (3 ml) at 0°C for 30 min. The enzymes and metabolites of mitochondria were liberated and assayed (Wattiaux and de-Duve, 1956 and Astawrov, 1974). Cytochrome-c content of mitochondria was determined spectrophotometrically according to the method of Williams and Thorp (1969). Cytochrome-c-oxidase activity of spectrophotometrically according to Smith (1955) and succinate cytochrome-c-reductase activity of mitochondria was assayed spectrophotometrically as described by King (1963). Total soluble protein was determined as Lowary method (1951). Statistical analysis of the all biochemical analysis results were done by t-test (p < 0.05) according to Sendecor and Cochran (1967).

RESULTS AND DISCUSSION

It is well known that oxidative stress plays a major role in the etiology of diabetic complication and development of diabetes. ATP, ADP and AMP levels and the myokinase activity as affected by diabetes and the present hypoglycemic agents treatments of wild plant traditionally used in Egyptian folk medicine against diabetes (Anastatica hierochuntica and Cleome chrysantha) in liver, brain and kidneys tissues of male albino rats were determined and the results are given in table (1-2). The ATP levels were significantly elevated in the present study treatments of plants relative to diabetic control, these elevations due to ingestion of the both plant extracts into diabetic rats. The increased levels by ingestion of methanolic extracts of both plants were higher and significant than that recorded for the ether extracts which was significant alteration. The highest value of ATP was recorded for liver and brain

tissues. Kidneys tissue had the lowest value of the three organs tissues. In addition, the methanolic extract of Anastatica hierochuntica was more effective than that of Cleome chrysantha in the same conditions of diabetes. In connection, both ADP and AMP levels of the three organs tissues were reduced in contrary to ATP levels under the same effects of treatments in diabetic rats. The reductions of ADP levels was observed for methanolic extracts treated diabetic rats and was approximately similar to that of AMP levels relative to diabetic control. The high and significant influences of methanolic extract the both plants may be due to that extracts was rich in polyphenols which associated with increased with resistance of plasma constituents to oxidation and with an increase in plasma antioxidant capacity (Utsunomiya et al., 2005). The improved values of energy metabolites were still lower than that of normal control for ATP but still higher than that of normal control for ADP and AMP levels of brain, liver and kidneys tissues. The present results of energy metabolites (ATP, ADP and AMP) may be to either the high rate of ATP synthesis or the energy liberation during the metabolic processes through trapping phosphate with AMP and ADP (Murray et al., 2006), these findings were in parallel with these of the carbohydrate metabolism (previous study) (Abdel-Rahim et al., 2005) and respiratory system (in the present study). They found that alcoholic extracts of the both plants (rich in polyphenols) ingestion stimulated glycolytic processes in diabetic rats. These were not observed under the treatments of ether extracts ingestion (rich in lipid fractions). The obtained results led to the suggestion that at any circumstance associated with diminished availability of the prime dietary source of energy, namely carbohydrate, would accentuate the utilization of fatty acid for this purpose. In this respect, the stimulation of glycolytic metabolism and respiratory systems led to the accumulation of ATP that is rapidly utilized in protein biosynthesis processes by stimulation of adenylated cyclease activity to form cAMP (Elliott and Elliott, 2001).

Results showed stimulation in myokinase activity of brain, liver and kidneys tissues of diabetic rats (table 2). The ingestion of the present plants extract (ether and methanol) inhibited the activity relative to diabetic control. The inhibition of myokinase activity by ether extracts was low and insignificant, but that for methanolic extracts was high and significant. Generally, the increase in ATP contents due to the methanolic extracts ingestion into diabetic rats was mainly attributed to the influence of these rich polyphenol nutrients on the respiratory system (results of the present study).

The maintenance of organs tissue is likely accomplished through stimulation the glycolytic processes. The inhibition of myokinase activity in diabetic rats induced by methanolic extracts of the both plants may be due to ample ATP (Murry *et al.*, 2006). Myokinase catalyzed as the following reaction:

The reaction was stimulated after the complete utilization of ATP. Also, the amount of AMP might be due to the cyclic AMP which was formed from ATP to catalyze the adenylate cyclase reaction as follows:

Table (1): Plant extracts influences on the energy metabolites in albino rats organs tissues:

			ATI					AD)	AMP									
l		(Umol/g.:	tissue)				(µmol/g.	(µmol/g,tissue)									
Treatment	Brain		Liver		Kidneys		Brain		Liver		Kidneys		Brain		Liver		Kidneys	
	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%
Normal	7.60		11.01	_	2.01		0.61		0.17	,	0.16		0.31		0.69		0.25	
i	±	100	±	100	±	100	±	100	±	100	±	100	±	100	±	100	\ ±	100
control	0.20		0.90		0.09		0.036		0.011		0.009		0.014		0.031		0.016	1
Diabetic	4.00		6.70		1.22	_	0.85		0.21		0.20		0.41		0.76		0.29	
,	±	52.6	±	60.9	±	60.7	±.	139.3	±	123.5	±	125	±	132.2	±	110	±	116
control	0.27*		0.34*		0.05*		0.041*	L_	0.013*		0.010*		0.020*		0.016*	L	0.015*	
Ether	4.11		6.66		1.20		0.83		0.20		0.21		0.40		0.76	T	0.30	
extract of	±	54.1	±	60.5	±	59.7	±	136.1	±	117.7	±	131.3	±	129	±	110	±	120
Anastatica	0.27*		0.32*		0.06*		0.050*		0.012*		0.011*		0.019*		0.020*		0.014*	
Methanol	5.50		9.00		1.70		0.68		0.17		0.16		0.34		0.72		0.26	
extract of	±	72.4	±.	81.7	±	84.6	±	111.5	±	100	±	100	±	119.6	±	104	±	104
Anastatica	0.22*		0.47*		0.08		0.032		0.012		0.008		0.017		0.016		0.013	
Ether	4.50		6.71		1.20		0.84		0.21		0.19		0.41		0.77		0.28	
extract of	±	59.2	±	60.9	±	59.7	±	137.7	±	123.5	±	118.8	±	132.2	±	112	±	112
Cleome	0.26*		0.34*		0.05*		0.041*		0.013*		0.010*		0.020*		0.018*		0.015*	
Methanol	5.00		8.80		1.50		0.72		0.17		0.16		0.36		0.70		0.25	
extract of	±	65.8	±	79.9	±	74.6	±	118	±	100	±	100	±	116.1	±	101	±	100
Cleome	0.29*		0.41*		0.04*		0.036*		0.011		0.007		0.014	<u> </u>	0.017		0.020	$oxed{oxed}$

[%] Relative to normal control

^{*} Values are significantly different with normal control P < 0.05- Each value represents the mean of 6 rats (Mean \pm SD)

Table (2): Plant extracts influences on myokinase activity in mitochondrial and cytoplasmic fractions of albino rats organs tissues:

		Mitoc	hondrial myd Imol ADP/m	kinase ac	tivity	Cytoplasmic myokinase activity (µmol ADP/mg protein)							
Treatment [Brai	n	Liver		Kidneys		Brai	n	Live	r	Kidneys		
	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	
Normal control	0.41	1	0.34		0.26		0.51		0.44		0.21	T	
	± 0.023	100	± 0.016	100	± 0.014	100	± 0.020	100	± 0.025	100	± 0.012	100	
Diabetic control	0.46	112.2	0.41	120.6	0.29	111.5	0.60	117.6	0.53	120.5	0.31		
	± 0.023*		± 0.019*		± 0.017*		± 0.027*		± 0.023*		± 0.015*	147.6	
Ether extract	0.45	109.8	0.40	117.6	0.30	115.4	0.61	119.6	0.53	120.5	0.30	}	
of Anastatica	± 0.020*		± 0.018*		± 0.013*		± 0.030*		± 0.022*		± 0.020*	142.9	
Methanol	0.43		0.37	108.8	0.27	103.8	0.55	107.8	0.48	109.1	0.24	1	
extract of Anastatica	± 0.021	104.9	± 0.018		± 0.012		± 0.024		± 0.025		± 0.012	114.3	
Ether extract of Cleome	0.46 ± 0.020*	112.2	0.41 ± 0.020*	120.6	0.28 ± 0.015*	107.7	0.60 ± 0.024*	117.6	0.52 ± 0.026*	118.2	0.31 ± 0.012*	147.6	
Methanol	0.44		0.39		0.28		0.57	111.8	0.50	113.6	0.28		
extract of Cleome	± 0,027	107.3	± 0.020*	114.7	± 0.016	107.7	± 0.027		± 0.024		± 0.013*	133.3	

[%] Relative to normal control

^{*} Values are significantly different with normal control P < 0.05

⁻ Each value represents the mean of 6 rats (Mean \pm SD).

The extent of coupling oxidation to phosphorylation, evident in mitochondria provided a means by which the role of oxidation of foodstuffs by respiratory oxygen was regulated by high requirement of the cell for useful energy. The utilization of ATP to drive the energy requiring processes of the cell automatically increased the available supply of ADP and inorganic phosphate, which in turn became available to react in the coupling mechanism and permitted respiration to proceed (Chatterjea and Shinde, 2002). At the effect of methanolic extracts of the plants ingestion, the oxidative phosphorylation was stimulated due to the respiratory oxygen and inorganic phosphate that was increased by stimulation of phosphatases activity under the same conditions these were paralleled with the increase of ATP formation (Abdel-Rahim et al., 2005).

In case of respiratory system of cytochrome-c which connected with respiratory system of mitochondria and its enzymes which considered one of important markers of mitochondrial biosynthesis and turnover (Murry et al., 2006). Table (3) observed the results of cytochrome-c contents and the activities of cytochrome-c-oxidase and succinate-cytochrome-c-reductase in mitochondrial fractions of brain, liver and kidneys tissues. The levels of cytochrome-c of three organs tissues were reduced relative to normal control in diabetic rats. These values were returned to about normal level by ingestion of the both plants methanolic extracts, but still lower than those of normal control. These were not observed by treatments of plants ether extracts. Table (3) also, showed the activity of oxidative enzymes related to cytochrome-c in mitochondria. From these data, the diabetes stimulated the activities of mitochondrial cytochrome-c-oxidase. The present stimulated values were returned to about that of normal control animals by the ingestion of methanolic extracts of the both medicinal plants but not by their ether extracts. Ether extracts ingestion the present plants slightly but insignificant inhibited the enzyme activity of diabetic rats. The same trend was observed in case of succinate-cytochrome-c-reductase activity, but the effects of diabetes on the activity of succinate- cytochrome-c-reductase were more than that on cytochrome-c-oxidase. The activity values of the both enzyme were still more than that of the normal control in diabetic rats treated by the both extracts either of Anastatica hierochuntica or of Cleome chrysantha. The present finding suggested that diabetes damaged cytochrome-c in mitochondria, which is synthesized in extra-membrane of mitochondria and can be used as an important maker to evaluate the inner membrane of mitochondrial turnover (Murry et al., 2006).

The results concluded that diabetes caused a damage in mitochondria structure, however the influences of methanolic extracts on diabetes was as antagonistic agent (antioxidant). In addition, the stimulation of cytochrome-c-oxidase and succinate-cytochrome-c-reductase activities of diabetic animals may be due to not only elevation of respiratory system activity but also stimulation in the oxidative enzymes activity which used succinate and cytochrome-c as metabolites. The present results are in agreement with that reported by Utsunomiya *et al.* (2005) and Ismail *et al.* (2007) and, they found that the polyphenol extracts (by methanol) reduced the toxic and stress of diabetes on mitochondrial respiratory system.

Table (3): Plant extracts influences on cytochrome-c respiratory system in albino rats organs tissues:

Treatment	C,	me-c-ox nol/min/		Succinate-cytochrome-c-reductase activity ([Imol/min/g tissue)							Cytochrome-c contents (µmol/g tissue)							
	Brain		Liver		Kidneys		Brain		Liver		Kidneys		Brain		Liver		Kidneys	
	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%	Value ± SD	%
Normal control	51.00 ± 3.33	100	16.06 ± 1.00	100	15.00 ± 0.88	100	30.12 ± 1.55	100	2,22 ± 0.12	100	3.27 ± 0.16	100	9.11 ± 0.06	100	23.00 ± 1.12	100	27.19 ± 1.78	100
Diabetic control	61.00 ± 3.47*	120	18.06 ± 0.92*	113	16.12 ± 0.91*	108	54.11 ± 2.22*	179.6	4.10 ± 0.21*	185	4.61 ± 0.20*	141	7.00 ± 0.31*	76.84	18.22 ± 0.82*	79.22	17.89 ± 1.11*	65.8
Ether extract of Anastatica	60.11 ± 3.21*	118	18.00 ± 0.84*	112	16.00 ± 0.82*	107	54.20 ± 3.00*	179.9	4.00 ± 0.19*	180	4.42 ± 0.21*	135	7.11 ± 0.41*	78.04	18.01 ± 0.90*	78,30	17.70 ± 1.00*	65.1
Methanol extract of Anastatica	53.11 ± 3.00	104	16.87 ± 0.77	105	15.16 ± 0.80	101	37.21 ± 1.61*	123.5	2.97 ± 0.19	134	4.00 ± 0.17	122	8.00 ± 0.40	87.82	20.13 ± 0.99	87.52	22.22 ± 1.21	81.7
Ether extract of Cleome	59.89 ± 3.01*	117	18.10 ± 0.81*	113	16.01 ± 0.74*	107	54.00 ± 3.00*	179.3	3.98 ± 0.20*	179	4.50 ± 0.22*	138	7.02 ± 0.31*	77.06	18.20 ± 0.97*	79.13	17.60 ± 1.00*	64.7
Methanol extract of Cleome	54.61 ± 3.04	107	17.11 ± 0.90	107	15.60 ± 0.76	104	40.00 ± 1.88*	132.8	3.04 ± 0.17	137	4.22 ± 0.22*	129	7.89 ± 0.30*	86.61	20.00 ± 0.95	86.96	20.11 ± 0.99	74.0

[%] Relative to normal control

^{*} Values are significantly different with normal control P < 0.05- Each value represents the mean of 6 rats (Mean \pm SD)..

In addition, the previous study (Abdel-Rahim et al., 2005) found that the oxido-reductase enzymes could enhance the metabolites oxidations such as pyruvate and succinate in which via cytochrome-c mechanism, overcome the initial lack of oxygen and provided the animal with energy requirement. This stimulation in the animal tissues could be due to the physiological status of animals (Yin et al., 2006). On the other hands, Utsunomiya et al. (2005) and Ismail et al. (2007) added that the polyphenol extracts increased plasma adiponictin content, these observation suggest that improvement of insulin sensitivity with polyphenol extracts may be mediated partly through increased adiponictin.

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تحسين أنظمة الطاقة لفتران مصابة بمرض السكر باستخدام نباتى كف مريم والسموة

امام عبد المبدى عبد الرحيم ، حسام الدين سعد البلتاجي ، شريفة حسين صلاح ، ، رامى محمد عبد الحميد روميلة ،

- قسم الكيمياء الحيوية كلية الزراعة جامعة القاهرة جيزة مصر
 - ** قسم الخلية الحيوية المركز القومي البحوث القاهرة مصر

تم دراسة التأثير الخافض لعمكر الدم لكلا من نباتى كف مريم والعموة على الذكور الفئران البالغة والمصابة بمرض العمكر نتيجة المعاملة بمادة العتربتوزيتوسين. هذا وقد تم حقن كلا من المستخلص الكحولى والإيثيرى لكلا النباتين لحيوانات التجارب المصابة بمرض العمكر لدراسة تأثيرهم على أنظمة تمثيل الطاقة والسيتوكروم جلى في كل من المخ، الكبد والكليتين. وقد لوحظ في تمثيل الطاقة للمجموعة الكنترول للفئران المصابة بمرض العمكر انخفاض في محتوى الأدينين ثلاثي الفوسفات وزيادة في محتوى كلا من الأدينين أحادى وثنائي الفوسفات. كما لوحظ بالإضافة لذلك حدوث

ارتفاع فى نشاط انزيم الميوكينيز. هذا وقد سببت التغذية على المعستخلص الأيثيسرى لكلا النباتين تغيرات غير معنوية فى تأثير مرض السكر على محتوى كلا من (الأدينين أحادى، ثنائى، ثلاثى الفوسفات) ونشاط انزيم الميوكينيز.

هذا وقد أحدثت تغنية الفئران المصابة على المستخلص الكحولى لكلا من كف مريم والسموة ارتفاع معنوى في مستوى الأدينين ثلاثي الفوسفات وانخفاض في كللا من الأدينين أحادى وثنائي الفوسفات. بينما حدث انخفاض في نشاط انزيم الميوكينيز.

بالإضافة لحدوث انخفاض فى محتوى السيتوكروم جوريادة معنوية فى نشاط انزيمى السيتوكروم جوا وكسيديز والسكسنات جريدكتيز. وقد أثبتت نتائج هذه الدراسة ان تغذية الفئران على المستخلص الأيثيرى لكلا النباتين قد أحدثت تغيرات غير معنوية فى تأثيرات مرض السكر على نظام السيتوكروم جوالي النقيض فالتغذية بالمستخلص الكحولى لكلا من نباتى كف مريم والسموة قد احدثت تاثيرات معنوية كبيرة فى نظام السيتوكروم جوالخاص بالفئران المصابة بمرض السكر وساهمت فى تحسين أنظمة النتفس وجعلتها مماثلة الفئران السليمة.