Research Journal of Applied Sciences 2 (7): 832-838, 2007

Effects of Dietary Colocynthis and Sunflower Fatty Acids Containing Oils on Lipid Metabolism and on Antioxidant Parameters in Streptozotocin-Induced Diabetic Rats

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Abstract: Oxidative stress plays a role in cardiovascular dysfunction. This is of interest in diabetes, a clinical conditions characterized by oxidative stress and increased previous of cardiovascular disease. It's the leading causes of death in people with diabetes type II (non insulin dependent)or NIDDM. This disease is very common metabolic disorder affection of hyperglycaemia and insulin resistance suppose by important roles of dyslipideamia, (cause of peroxydation, so increase free radicals and damage. So the optimal diet for persons with diabetes has long been a subject of controversy. Dietary therapy was the only treatment available in the era before insulin therapy. The goal of nutrition and medical therapy in diabetes is to prevent complication by maintenance of glycaemia control and other parameters like lipoproteins and cholesterol. Nutritionally beneficial compounds naturally present in vegetables seeds are an original source of fats and fats soluble vitamins, phytosterols ect… both important in human diet and to prevent complications, so in this idea we use *Citrullus colocynthis* as source of oils (this plant is known by numerous therapeutically properties, is used by the diabetic population NIDDM) in isocaloris diet in induced streptozotocin diabetic male Wistar rats. In this study, we evaluate in the first, the effect of this oil compared with sun flower on glucidic, lipidic metabolim and on hepatic activity, so we measured levels of glucose, insulin, Cholesterol, Triglycerides, NEFA, C-LDL, C-HDL in plasma, glutathione and Malondialdehyde (MDA) in liver homogenates of the rats fed a diet with the different oils. Our results show clear correction of atherogene parameters (CT,TG,NEFA and C-LDL) in diabetics rats versus non diabetics rats,an increase of the glutathione level and decrease of MDA levels in diabetic rats fed with diet 2. Colocynthis oil diet increased the diminsched vitamin A levels in diabetic rats and vitamin C concentrations. Also, this improved the decreased erythrocyte superoxyde dimutase and glutathione peroxidase activities in diabetic.

Key words: Non Insulin Dependant Diabetes (NIDDM), citrullus colocynthis oil diet, antioaxydant status, vitamins

INTRODUCTION

The interest in disturbances in oxidative stress usually observed in patient with type II diabetes rely on the links between these disturbances and diet (apparition of cardiovascular complications) (Diebolt *et al*., 2001; Giuspina *et al*., 2003; Rouchy, 2001).Type II diabetes (Non Insulin-dependent Diabetes Mellitus) or NIDDM is a major care problem because of the high frequency of this disease. The percentage of the population is even grater. Abnormal insulin response to glucose and insulin resistance of target peripheral tissues (liver, Muscle and adipose tissue) are the major features observed in type II diabetes (Vollenweider and Scherrer, 2001). Conversely, in NIDDM disease, a decrease in B cell volume and a reduction in B cell mass correlate with impairment of glucose induced insulin secretion, age of onset of diabetic population appears be important determinant in the development of the disease, sedentary life style and excessive intake of saturate fat, has also been implicated for elevation of the incidence of this disease(Chanson *et al*., 1991)however, a lot of arguments improved that Nutrition is Important for Treatment of Diabetic patient (NIDDM) (Monnier *et al*., 2006). So, the

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most important metabolism are seriously affected, specially glucidic and lipidic metabolism (peroxydation and increase free radicals) (Battula *et al*., 2000; Davis, 1999). In fact, experimental studies have demonstrated that dietary intake of vegetables oils is recommended, because unsaturated fatty acids containing which favourite the diminution of rise plasmatic atherogenic parameters(Cholesterol and Triglycerides) free or linked to Low Density Lipoproteins (TC,TG,C-LDL) and in the same time decrease of oxidative stress and increased of antioxidative status (Battula *et al*., 2000; Davis, 1999). induced a protector role to prevent major complications (CHD and thrombosis)(Grundy, 1999). phytosterols containing oils play the same role(protection), in fact we note the presence of antioxidant agent like polyphénols and vitamin E (Delplanque, 2002). In this goal, our study is ported on the effects of colocynthis oil because its polyunsaturated fatty acids containing and the numerous therapeutics properties this plant, *Citrullus colocynthis has* been used for therapeutically advantages, is has also been suggested that it has antidiabetics properties in some Mediterranean countries (Zyyat *et al*., 1997). In Algeria, diabetic subjects currently treat their disease with oral infusion of colocynthis fruit. The phytochemical investigation of this treat their disease with oral infusion of colocynthis fruit. The phytochemical investigation of this fruit has been a subject on interest to many authors, but a few studies were done for colocynthis oil, so from this note we study the effect of this oil on the streptozotocin induced diabetic rats in order to found an hypoglycemic effect at least reduced diabetes complication as CHD and thrombosis and on antioxidant status sand lipid metabolism in diabetic and bon diabetic rats.

MATERIALS AND METHODS

Plant description: Fresh or dry ripe *Citrullus colocynthis* were collected from Mecheria «west of Algeria». The fruits were sliced in half and the seeds manually removed mature black seeds were selected, the pulp removed and then they were ground into a powder with moulinex coffee grinder prepared for extraction. The lipid fraction was extracted with petroleum ether (40-60°C in a Soxlete apparatus for 06 h in laboratory (produits naturels" Tlemcen, Algeria).The solvent was evaporated and the lipid fraction residues were weighed, oil content in seeds was 17% (Sun flower oil is a commercial cooking product, Cevital).

Component acids analyses were done by gas chromatography HPLC apparatus: Chromatographic analysis was carried using a model of Triathlon Spark Holland (Beckman) auto sampler, a model 218 quaternary pump (Beckman), a model Shimadsu RF-551 fluorescence detector and a model BD 112 (Kipp and Zonen) recorder. Separation of fatty acids was achieved in a reversed phase Kromasil C18 column (5 μm, 250 × 4.6 mm i.d). The composition of the mobile phase changed following a gradient programme from MeOH-H2O (92:8) to MeOH-H2O (100:0) in 25mn at 1,7 $mL^{-}1$ mn. The selected excitation and emission wavelengths were 325 and 395 nm, respectively. Composition of fatty acids oils is shown in Table 1.

Animals diet: Male Wistar rats weighing 80± 5g ((1month old) were housed in wire botton cages with a 12 h light/dark cycle. Food and water were given ad libittum. Food was replaced daily with any uneaten portion discarded. The rats were maintained at 24°C and constant humidity 60%. Each group of rats was divided into two groups of five rats each and fed different diets for 2 months:

Group 1: (Non diabetic rats) rats who received a diet with (16% casein + 8% sun flower oil)(diet 1).
Group 2: (Diabetic rats) rats who received a diet with (diet 1).
Group 3: (Non diabetic rats) rats who received a diet with (16% casein + 8% colocynthis oil)(diet 2).
Group 4: (Diabetic rats) rats who received a diet with (diet 2).

Composition of diet is shown on Table 1.

Induction of experimental diabetes: Diabetes was induced by intraperitoneal injection of streptozotocin (sigma Aldrich) (C8H15N3O7) dissolved in 0.1 M cold sodium control buffer (Ph = 4.5) at a dose of 65mg kg^{-1} Body Weight (BW). (The injection of STZ has been done after 3weeks of diet introduction).

Table1: Composition of the diet

Constituents (g^{-1}100g diet)	Sunflower oil diet(diet1)	Colocynthis oil diet(diet2)	Energetic values (Cal/KCAL)
Casein	16	16	64
Methionine	0.3	0.3	1.2
Starch	55.7	55.7	222.8
Saccharose	05	05	20
Cellulose	05	05	/
Mineral mix	7.37	7.37	/
Vitamin mix	02	02	/
	20.5 % SFA	**17 % SFA**	
	63.5 % MUFA	**8.7 % MUFA**	
Oil	08 **16 % PUFA**	08 **74.3 % PUFA**	72
Total	100	100	380

Mineral mix provided the following nutrient (g100g^{-1} of dry diet) : Ca,4; K,2.4; Na,1.6; Mg,0.4 Fe,0.12; elements (traces): Mn,0.032; Cu, 0.05; Zn,0.018. Vitamin mix provided the following nutrient (mg/1Kg of dry diet): retinol, 1.8 ;Cholicalciferol, 0.019; thiamine, 6; riboflavin, 4.5; pantothenic acid,21; inositol;45; ascorbic acid,240; á tocopherol,51; nicotinic acid, 30;folic acid, 1.5; biotin, 0.09

Surgery: After 12 weeks (08 weeks dietary period) rats were deprived of food for twelve hours(12H)) and than anesthetised by an intraperitoneal (i.p) injection of pentobarbital (10%) at dose of 200 μL^{-1} 100g. B.W. They were there placed on a thermo stated table to keep them at a constant temperature. Blood was collected from the abdominal aorta into tubes with EDTA.

The plasma was obtained by low speed centrifugation (3500 t^{-1} min) and was immediately used for biochemical analysis. After liver was removed immediately, rinsed with cold physiological saline solution (NaCl).

Biochemical parameters: Blood glucose levels were measured by a reflective glucometer(model GX,Ames Miles Bayer diagnostics) using the glucose oxidase method (glucostix strips: Bayer diagnostics).

Insulin concentrations were measured in serum by radioimmunoassay method using a Beta matic conter. Plasmatic cholesterol, NEFA, triacylglycerol and LDL-C levels were measured by enzyme kit (Boeringer, Meylan, France). LDL and HDL fractions were obtained by precipitation.

Liver dosages: The dosages of MDA and the glutathione were performed in fragments of cool liver on different rats fed with diet1 and 2.

Dosage of the Malondialdehyde (MDA): The MDA is one of the terminal products, formed at the time of the decomposition of the Polyunsaturated Fatty Acids (PUFA) mediated by the free radicals. The dosage of the MDA was produced by using 1 g of liver, which was added to 3 mL of Kcl solution (1.15M); and then was grinded by a homogenizer of DOUNCE (KONTES; Glass company year ISO-9001 steered firm, New Jersey, USA). Consequently, wedded 0.5 mL of trichloracetic acid 20% and 1 mL of Thiobarbituric Acid (TBA) 0.67% into 0.5 mL of the homogenate. The mixture was heated to 100°C for 15 min and after its cooling, we added 4 mL of butanol. After centrifugation to 3000 $tours^{-1}$ min for 15 min, the optic density was determined on the remaining to the spectrophotometer (LKB II) at 530 nm. The MDA activity is expressed in UI^{-1} gr of liver.

Dosage of the hepatic glutathione: One grammar of liver (cool or frozen) was homogenized in 3 volumes of Trichloro-acetic Acid (TCA) 5% together with a homogenizer of DOUNCE. Homogenized and centrifuged to 2000 trs^{-1} min. Then, 50uL of the remaining were diluted in 10 mL tampon phosphate 0.1 M, pH = 8. Consequently, we added 20uL of DTNB (dithiobis nitro-benzoic acid) 0.01M to 3 mL of the dilution mixture. The measurement of the optic density was performed at 412 nm against a control prepared in the same conditions using TCA 5%. The concentrations are expressed in mmoles of glutathion/gr of liver. Theyare deducted from a range of glutathione, which was prepared with the same conditions as dosage did.

Preparation of the range:

GSH 0 mM : 50 µL H2O (distilled water)
GSH 1.25 mM: 12.5 µL of the solution of GSH to10mM + 100 µL H2O
GSH 2.50 mM: 25 µL of the solution of GSH to10 mM + 25 µL H2O
GSH 5 mM : 50 µL of the solution of GSH in 10mM+25 µL H2O
GSH 10 mM : 50 ML of glutathione 10 mM (3.15 mg of glutathione in 10 mL H2O distilled water).

Determination of erythrocyte antioxidant enzymes activities:Glutathione peroxydase: Glutathione peroxydase (GSH-Px;1.11.1.9) was determined according to the method of Paglia and Valentine (1967) using cumene hydroperoxydase as a substrate. One unit of GSH-Px activity is defined as the amount of enzyme that gives a 90% in glutathione concentration. Glutathione reductase (GSSG-Red; EC1.6.4.2) activity was evaluated at 340 nm by measuring the decrease in absorbance of nicotinamide adenine dinucleotide phosphate (reduced) in the presence of oxidized glutathione (Goldberg and Spooner, 1992). The unit of enzyme activity was defined as the amount of enzyme which oxidized 1 mMol of NADPH per minute.

Superoxide dismutase (SOD;EC1.15.1.1) activity was measured at 412nm the NADPH oxidation procedure (21) and expressed as units of SOD per gram HB.

Determination of vitamin A and E levels in plasma: Plasma á tochopherol (vitamin E) and retinol (vitamin A) were determined by reverse phase HPLC (Zyyat *et al*., 1997). The stationary phase constituted greffed silica (C18 column, HP ODS hypersil C18; 200×4.6 mm; Lara spiral, maintenance temperature of analytical column, 35°C)the mobile phase was a mixture of methano/water (98/2,v/v) at a flow rate of 1 mL^{-1} min. vitamins were extracted by hexane, dried under nitrogen and resuspended in methanol. The extracted vitamins were injected in the HPLC system (Zyyat *et al*., 1997). The HPLC peaks were detected by UV detector at 292 nm for vitamin E and at 325 nm for vitamin A. Representative chromato-grams were obtained by injection standard solutions.

Determination of plasma vitamin C level: Vitamin C levels were determined in plasma using the method of Roe and Kuether 1943. After protein precipitation with 10% trichloroacetic acid centrifugation, the supernatant (500 μL) was mixed with 100 μL of DTC reagent (9N sulphuric acid containing 2.4 dinitrophenylhydrazine 3% thiourea 0.4% and copper sulphate 0.05% and incubated at 37°C for 3H. after the addition of 750 μL of 56% (v^{-1} v) sulphuric acid, the absorbency.

Statistical analysis: Results were expressed as mean±Standard Error of Mean (SEM). The significance of differences between the mean of treated and control groups were established by student test"t". P-values less than 0.05 were considered to be significant.

RESULTS

Body, relative liver weights: Table 2 shows body, relative liver weights level of glycaemia for the diabetic rats and non diabetic rats fed with the 2 vegetables oils (colocynthis, sun flower).

The body weigh at the start and at the end of the experiment were significantly lower in diabetics rats fed with different diet (1, 2) compared with non diabetic rats fed with the same diet, respectively. However, liver relative weigh is significantly higher in diabetics rats fed with diet 1 versus non diabetic rats.

Plasmatic concentrations of glucose, insulin, total cholesterol, triacylglycerol, non asteriated fatty acids, LDL-cholesterol and HDL-cholesterol: The values of plasma glucose, insulin triacylglycerol, NEFA, total cholesterol LDL-C and HDL-C values are shown in the Table 2.

During experimental phase, we observed that the blood glucose levels of the diabetics were higher in diabetic group fed with different diet,but colocynthis oil diet diminished glycaemia in diabetics rats comparedwith diabetic rats fed with diet 1, but insulin levels decreased in the diabetics rats fed with diet 2 (colocynthis oil). In contrast triacylglycerol level is significantly lower in diabetic rats fed with diet 1 that non diabetic rats fed with the same diet, but is significantly higher in diabetics rat fed with diet 2 than non diabetics rats fed with diet 2.

However, the NEFA level is significantly decreased in diabetics rats fed with different diets than non diabetics rats fed with the same diets, respectively. On the other hand, diabetes induced an increase in total cholesterol levels in diabetic rats fed with diet 1 and 2 compared with their control fed with the same diet. The colocynthis oil diet induced a significant decrease in total cholesterol in diabetic rats. However, this value remained significantly lower than diabetics rats fed with diet 1. the plasma cholesterol level and C-LDL is decreased in diabetics rats compared to non diabetic rats fed with different diets but the level of C-HDL is unchanged in diabetics rats compared with non diabetics rats fed with diet (1,2).

Variation of the hepatic Malondialdehyde and Glutathione Concentration (MDA): The alterations in MDA and glutathione concentrations are depicted in the Table 3. We have noted an significant increase in MDA concentrations in the group of the diabetics rats fed with diet (1and 2)compared with non diabetic rat fed with diet (1 and 2), respectively. We have noted a reduction in glutathione concentrations in all diabetic groups fed with diet (1,2) compared with their controls fed with the same diet, respectively.

Table 2: Body weight, relative liver weight, glycaemia, insulineamia, triglycerides, cholesterol, C-LDL and C-HDL in diabetics and non diabetic rats fed with different diets

	Non diabetic rat (Diet 1) N = 5	Diabetic rat (Diet 1) N = 5	Non diabetic rat (Diet 2) N = 5	Diabetic rat (Diet 2) N = 5
Initial BW(g)	74.00±2.73	74.00±2.73	72.00±1.36	73.00±1.36
Final BW(g)	246.10±1.32	172.6*±3.45	244.13±1.40	186.8*±13.67
Glycaemia (gL^{-1})	0.78±0.12	2.47*±0.21	0.74±0.05	1.3*±0.14
Insulineamia (gL^{-1})	218.±1.00	151.*±5.00	144.±3.7	151.*±6.8
Triglycerides (gL^{-1})	0.39±0.03	0.53±0.11	0.53±0.02	1.23±0.22
Cholesterol(gL^{-1})	0.63±0.08	0.76±0.04	0.36±0.03	0.47±0.02
C-LDL (gL^{-1})	0.18±0.03	0.30±0.01	0.16±0.03	0.15±0.03
C-HDL (gL^{-1})	0.42±0.02	0.38±0.02	0.24±0.02	0.26±0.02
NEFA (gL^{-1})	0.51±0.17	0.35±0.05	0.20*±0.03	0.40*±0.11
Relative liver ($g100g^{-1}$ BW)	3.58±0.38	5.02±0.43	3.24±0.16	3.27±0.57

Results are expressed as mean ±standard error. * are statically significant in the diabetics rats versus non diabetics rats fed with the same diet

Table3: Variation of hepatic malondehaldehyde and glutathione concentration in diabetics and non diabetics rats fed with diet1 and 2

	Non diabetic rats (diet1)(n=5)	Diabetic rats (Diet1) STZ (n=5)	Non diabetic rats (diet2) (n=5)	Diabetic rats (Diet2) STZ (n=5)
MDA(n $moleg^{-1}$)	35.92±0.58	38.64*±0.36	46.95±2.01	51.14*±0.72
Glutathione($mmoleg^{-1}$)	0.9±0.06	0.75±0.05	0.98±0.03	0.8±0.04

Results are expressed as mean ±standard error. * are statically significant in the diabetics rats versus non diabetics rats fed with the same diet

Table 4: Antioxidant enzymes activities in erythrocytes(u/gHb) in diabetics and non diabetics rats fed with diet 1and 2

Enzymes	Non diabetic rats (diet1) (n=5)	Diabetic rats (Diet1) STZ (n=5)	Non diabetic rats (diet21) (n=5)	Diabetic rats (Diet2) STZ (n=5)
SOD u/gHb	858.38±6.93	793.24*±3.62	403.98±3.76	206.1*±9.08
GSSG-red U/gHb	84.4±1.85	56.04*±1.56	89.36±2.45	74.62*±2.59
GSH-PX U/gHb	133.24±1.9	67.20*±2.31	243.32±2.30	198.02*±1.4

Results are expressed as mean±standard error. *are statically significant in the diabetics rats versus non diabetics rats fed with the same diet

Table 5: Plasma vitamin C, vitamin E and vitamin A concentrations in diabetics and non diabetics rats fed with diet 1 and 2

	Non diabetic rats (diet1) (n=5)	Diabetic rats (Diet1) STZ (n=5)	Non diabetic rats (diet2) (n=5)	Diabetic rats (Diet2) STZ (n=5)
Vit A(μg mL^{-1})	16.±1.32	12.*±0.6	10.±1.08	7.94*±0.75
Vit E(μg mL^{-1})	8.7±0.9	11.6±0.54	9.19±0.96	15.08±0.62
Vit C(μg mL^{-1})	17.03±2.13	14.27*±2.32	18.8±1.2	16.22*±1.3

Results are expressed as mean ±standard error. *are statically significant in the diabetics rats versus non diabetics rats fed with the same diet

Antioxidant enzyme activities: Diabetes mellitus diminished SOD activity in both rats fed with diet 1 and rats fed with diet 2 (Table 4). The colocynthis oil diet induced a significant increase in SOD activity in control and diabetic rats (Table 4).

GSSG. Red activity was diminished significantly in control diet fed diabetic rats. The colocynthis oil diet induced a same effect in the activity on this enzyme. Diabetes mellitus caused a significant decrease in GSH-PX activity. Colocynthis oil diet induced also a significant decrease in the activity of this enzyme in diabetic rats (Table 4).

Plasma vitamin A and E concentrations: Plasma vitamin A concentration, diminished in diabetic rats fed with diet 1, were nomalized by feeding colocynthis oil diet (diet 2) (Table 5). Compared to controls, diabetic rats fed with sun flower oil diet (diet 1)exhibited lowered plasma vitamin A concentration. In this group, feeding colocynthis oil diet enhanced and restored its concentrations. The levels of vitamin E were altered in diabetic animals. Similary, feeding colocynthis oil diets failed to influence plasma vitamin E levels.

Plasma vitamin C concentrations: Plasma vitamin C levels was not altered in diabetic rats compared to their control. Diatery colocynthis oil enhanced and improved plasma vitamin C concentrations in this group up to the level of controls (Table 5).

DISCUSSION

Oxidative stress is obtained when the pro-oxidant challenge over whelms the antioxidant defences. Oxidative stress may contribute to the inactivation and maintenance. Treatment with antioxidants, may prevent or reverse abnormalities associated with diabetes and it's complications. many studies have reported that dietary supplement such as antioxidant, vitamins and poly unsaturated fatty acids prevent or at least attenuate organic impairment originate by excess oxidative stress (Mari-José *et al*., 2001; Serhan *et al*., 2004). In addition, modification in the macronutrients composition of the diet is an intervention that has been advocated by some decreased saturated fat, sugar. Indeed, essential fatty acids pattern influences the physical properties of cell membranes(fluidity and permeability). The activity of membrane receptors, enzymes and ion channels and cell response to various stimuli through the production of secondary messengers (Khan *et al*., 2003). In this study, we investigated the effect of diet with colocynthis and sun flower oils on glucidic and lipidic metabolisms and antioxidant defence status in diabetic and non diabetic Wistar rats. The pathogenesis of insulin resistance, a central feature of type II diabetes mellitus is complex and incompletely understood, but environmental factors including excess nutrients and obesity play a major role. Once diabetes exits, both chronic hyperglycaemia and hyperlipidemia further aggravate the already impaired action and secretion of insulin. These adverse metabolic consequences of chronic hyperglycaemia and hyperlipidemia have been conceptualized as glucose toxicity and lipotoxycity which increase oxidative stress (Gravena *et al*., 2002; Loiout *et al*., 2002) and many study demonstrated that glycemic disorder, is one of the main risk factor for cardiovascular disease are associated with activation of oxidative stress (Monnier *et al*., 2006). Our results have shown that plasma glucose concentration were not significantly affected in diabetic rats fed with colocynthis oil diet so in this study we can say that vegetables oils specially Citrullus colocynthis oil are beneficial for diabetes regulation because of containing polyunsaturated fatty acids, monounsaturated fatty acids and perhaps for other components. Many studies have approved the benefice effect of these components of the correction of plasma glucose concentration in diabetes care (Delplanque, 2002; Gravena *et al*., 2002). However, our result show decrease plasma insulin concentration only in diabetics rats fed with diet (1.2), because their is an increasing evidence that fatty acids play a role in stimulus response coupling in the pancreatic beta cell (Freyse *et al*., 2003). Insulin resistant type II diabetes

mellitus is known to be associated with increased concentrations of plasma TG, NEFA and cholesterol (Cuvelier *et al*., 2004) but our result show a serious decrease of plasma total cholesterol, LDL-C, NEFA and TG and level of CHDL is unchanged in diabetics rat compared with non diabetics rats fed with different diet respectively, numerous studies have shown that elevated plasma LDL cholesterol and triacylglycerol concentrations are associated with accelerated atherosclerosis (Scaccini *et al*., 1992) and many studies have shown that vegetables oils have a beneficent role on these parameters (Ren and Okpala, 2005) and the other hand, Scaccini (1992) have suggested that LDL particles isolated from rabbit fed with a high vegetables oils were remarkably more resistant to oxidative stress (Jain *et al*., 1989) however, numerous metabolic studies have shown strong cholesterol lowering effects of vegetables oils rich in linolenic acid (Delplanque *et al*., 2002).

Oxidative stress has been suggested to be contributory factor in vascular complications of diabetes in various organs (Therond *et al*., 2000). So, the causal relationship between diabetes and oxidative stress has been substantiated by Jain *et al*. (1989) who found and increased peroxydation of membrane lipids an accumulation of malonedyaldehyde in lever of diabetic patients (Scaccini *et al*., 1992) but our results show an increase of MDA concentrations in diabetics rats compared with non diabetic rats but this increase is lower that the normal level and concerning glutathione, we observed a decrease of the concentration in diabetic rats versus non diabetic rats, but these decrease is non significant compared with the normal values.

Regarding the individual antioxidant, we have already found unaltered plasma levels of vitamin C, whereas plasma vitamin A and E contents were decreased in diabetic patient (Cao *et al*., 1993), vitamin E is the main liposoluble antioxidant. It scavenges peroxyl radicals produced during lipid peroxydation leading to tochopheroxyl radical while vitamin C plays a major role in regenerating vitamin E. Vitamin A has ability to react directly with reactive oxygen spices and is also considered as an antioxidant factor. It is well reported that oxidative stress induced by both the increases in free radicals and disturbance of the free radical scavenging system in diabetes mellitus (Sinclair, 1993).

So from these results, we can suggest colocynthis oil (which is original) have a benefit effect to attenuate the attack of free radicals and lipid peroxydation, because of containing of our oils (unsaturated fatty acids, antioxidant vitamins,phytosterol…).

CONCLUSION

Dietary recommendations continue to evolve as we gain a better understanding of the health effects of nutrients. Among several factors, related to life style habits that could influence cardiovascular risk the beneficial effect of diet has already been underlined in numerous of study.The optimal diet for persons with diabetes has long been subject of controversy. Dietary therapy was only treatment available in the era before insulin therapy.

The result of this study, demonstrate that citrullus colocynthis oil is beneficent for attenuating complications and perhaps to prevent from diabetes because of its polyunsaturated fatty acids and other nutrients like liposolubles vitamins, polyphénols, we observed a normal glycaemia and malonedialdehyde concentrations in diabetics rats.

Thus, colocynthis oil consumption influences beneficially the blood glucose, triglycerides, cholesterol NEFA, lipid peroxidation and antioxidant levels in diabetic rats.

Finally, we suggested other studies in this way in order to develop other effects of this oil and its importance for human use.

ACKNOWLEDGEMENT

The authors would like to thank Claude Rouch for her technical assistance in determination of fatty acids compositions of oils by HPLC.

Laboratory of physiopathology of nutrition, Paris 7, Paris, France. Nassima mokhtari and Meriem saker for their help to determinate some parameters. Laboratory of Physiology, University of Tlemcen Algeria.

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