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Comparative analysis of the protective effect of melatonin and *Cleome droserifolia* extract on antioxidant status of diabetic rats

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Abstract

The present study was carried out to compare the antihyperglycemic effects of melatonin and extract of *Cleome droserifolia* separately or in combination on the antioxidant defense system of diabetic rats. Melatonin (10 mg kg^{-1}) and *C. droserifo*lia (0.045 g $kg^{-1}day^{-1}$) were given i.p. after induction of diabetes with alloxan (110 mg kg^{-1}). Administration of alloxan to rats resulted hyperglycemia, hypoinsulinemia, reduced thyroid hormone levels and decreased body weight. These changes were accompanied with significant decrease in serum total protein, nitric oxide (NO), total antioxidant capacity (TAC), liver glutathione (GSH) level and catalase activity (CAT) of diabetic rats. Serum protein level of the melatonin or extract groups did not differ significantly during the period of the study as compared with the diabetic group, while it returned to the normal level in the case of combined-exposure. Melatonin increased the levels and activities of antioxidant markers including total protein, GSH, catalase and NO as compared with diabetic group. Melatonin and crude extract of *C. droserifolia* caused a significant decrease in liver tissue malondialdehyde (MDA) levels. Furthermore, treatment of diabetic rats with crude extract or its combination with melatonin had more potent effect on liver GSH level, insulin, T_3, T_4 and T_3/T_4 ratio than treatment with melatonin only. *C. droserifolia* was found to be less effective on CAT levels in liver than melatonin. TAC had positive correlation with GSH and CAT, while it had negative correlation with MDA. The present results confirmed that diabetes increased oxidative stress in liver and indicated the role of melatonin in combating the oxidative stress via its free radical-scavenging and antioxidant properties. Also, crude extract prevented any diabetic complications by reducing oxidative stress and protected rats from oxidative damage. Considering the much lower concentration of melatonin compared with extract, melatonin seems to be more potent antioxidant. Concomitant exposure to melatonin potentiates crude extract as antihyperglycemic effect on diabetic rats.

Keywords: *Cleome droserifolia*, anti-hypergycemic, melatonin, antioxidant, thyroid hormones, rat.

Introduction

Diabetes mellitus (DM) is a chronic metabolic condition characterized by disorder of glucose homeostasis. Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to excess formation of free radicals (FR) (Feillet-Coudray *et al.*, 1999 and Ceriello, 2003) and decreased activity of antioxidant defense systems (Ďuračková, 1999). Increased formation of FR in Type 1 and Type 2 diabetes mellitus can be a risk factor of the disease. It occurs as a result of two processes: i) decreased activity of the body antioxidant systems (Muchová *et al.*, 1999). ii) auto-oxidation of reducing saccharides and formation of adducts with proteins. Antioxidant levels in the blood and tissues are important factors of sensitivity of individual tissues to oxidation stress (Ďuračková, 1998). There are different classifications of antioxidants according to their mode of action; Chapple (1997) and Bonnefont-Rousselot *et al*. (2000) differentiated them into three main groups. i) Preventative antioxidants that prevent the formation of new reactive oxygen species (ROS) as caeruloplasmin, metallothioniene, albumin, myoglobin, ferritin and transferrin. ii) Scavenging antioxidants which remove ROS once formed,

thus preventing radical chain reactions. These include reduced glutathione (GSH), vitamine E, vitamin C, α-carotene, uric acid and bilirubin. iii) Enzyme antioxidants that function by catalyzing the oxidation of other molecules. This group includes superoxide dismutase that produces hydrogen-peroxide from superoxide radicals, glutathione reductase, glutathione peroxidase and catalase (CAT) which decompose hydrogen-peroxide (Trocino *et al*., 1995).

It has been suggested that oxidative stress plays a role in the development of diabetes and diabetic complications (King & Brownlee, 1996). The majority of free radicals and toxic molecules are derived from oxygen. The number of different antioxidant components in serum and tissues makes it relatively difficult to measure each antioxidant component separately. In addition, since there is a co-operation between various antioxidants, looking at one in isolation from rest may not accurately reflect their combined action. Therefore, the measurement of the total serum antioxidant capacity seems to represent a suitable biochemical parameter for evaluating the overall antioxidant status resulting from antioxidant intake or production and their consumption by the increasing levels of oxidative stress (Chapple, 1997).

The liver is one of the major sites of thyroxine (T_4; tetra-iodo-thyronin) metabolism (Bullock *et al*., 1991). It has been reported that several systemic non-thyroid diseases induce subnormal levels of serum tri-iodothyronine (T_3) and the T_3 / T_4 ratio. It has been suggested that low levels of T_3 and the T_3/T_4 ratio may be useful for the estimation of microsomal dysfunction (Itoh *et al*., 1988).

Melatonin, the chief secretory product of the pineal gland, where serotonin is converted to melatonin which was found to be a free radical scavenger and has a potent activity as an antioxidant (Montilla *et al*., 1998 and Kawanishi & Sakurai, 2002). Kumar *et al*. (1999) reported that melatonin administration lessened the harmful effect of nitric oxide via its indoleamine structure and provided protection against cyclosporine nephrotoxicity in rats. In addition, melatonin decreases lipid peroxidation and increases glutathione peroxidase in diabetic rats induced by streptozocin. Moreover, it prevented many diabetic complications (Vural *et al*., 2001 and Baydas *et al*., 2002) and protected liver and kidney cells (Kamel *el al*., 2002).

Cleome droserifolia, family Capparaceae (Boulos, 1999) grows in different regions of North Sinai, Egypt. It used for folk medicine, especially in Sinai for treatment of the persons suffering from non-insulin dependent diabetes (Ismael, 1992). It has hypoglycemic properties (Yaniv *el al*., 1987) as significantly supperssed the rise in peripheral blood glucose concentrations of albino rats (Nicola *et al*., 1996).

Based on these results of the antioxidant properties of melatonin and possible hypoglycemic activity of crude extract of *C. droserifolia*, a comparative study was undertaken between their effects on selected metabolic parameters and antioxidant status of alloxan-induced diabetic rats. The evaluation of the possible antioxidant activity of chronic administration of melatonin and *C. droserifolia* was carried out. In addition, the goal of this study was to determine interaction between the two treatments and whether the treatment of diabetic-induced rats would alleviate liver lesions caused by diabetic.

Materials and methods

Chemicals

Melatonin was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), alloxan was obtained from Aldrich Chemical Co and Tween-80 synthesis grade was purchased from Scharlaau Chemie S.A.

Plant extraction

C. droserifolia (Forssk.) was collected in Al-Arish, North Sinai, Egypt. The powdered leaves and small branches of *C. droserifolia* plant were extracted by cold percolation using 95% ethanol till exhaustion (Egyptian pharmacopoeia, 1984). The ethanol was evaporated under reduced pressure to give crude ethandic (EOH extract) . It was i.p. injected at a dose of

0.045 g $kg^{-1}day^{-1}$ (0.2 ml/rat) for 3 weeks that relevant to the save and effective dose on blood glucose as shown in the preliminary study.

Animals and Experimental Design

A total of 50 male Albino rats, *Rattus rattus*, weighing 120-140 g were housed in plastic cages with free access to water and food *ad libitum* for at least one week before the experiment. After one week of acclimatization, rats were randomly divided into 5 groups (10 rats/group) as follows. Group I, non-diabetic rats were injected with normal saline (0.2 ml) and considered as negative control. Group II was alloxan-induced diabetic rats and considered as positive control. Group III was alloxan-induced diabetic and melatonin-treated rats. The animals were daily intraperitoneally (i.p.) injected with 10 mg kg^{-1} melatonin in 1% ethanol-saline after 3 days of alloxan treatment. Group IV was alloxan-induced diabetic rats and received *C. droserifolia* extract (i.p) at a dose of 0.045 g $kg^{-1}day^{-1}$ after 3 days of alloxan treatment. The last group (V) was alloxan-induced diabetic rats and received melatonin and *C. droserifo* treatment in combination (1:1; V/V). There was not a vehicle group for melatonin group, because previous studies demonstrated that the solvent containing 30% or less ethanol did not influence diabetes (Montilla *et al*., 1998 and Sailaja-Devi *et al*., 2000). Diabetes was developed 3 days after i.p. administration of a single dose of 110 mg kg^{-1} body weight of alloxan, freshly prepared in distilled water, to 16 hours fasted rats (Benny *et al*., 2000). Three days after alloxan administration, blood glucose levels were measured in all animals. Alloxan-treated rats having a blood glucose level less than 180 mg dL^{-1} were excluded from the study.

Blood and liver sampling

Body weight of the experimental groups was recorded for each animal after one week of alloxan administration, and after three weeks (wk) beyond this period. Also, the ratio of organs weight to body weight was recorded.

Blood samples were collected from the medial retro-orbital venous plexus (Sanford, 1954) under ether anesthesia after 3 weeks of treatment. Blood sample was centrifuged at 3000 rpm for 15 mins. and serum was collected for different biochemical analyses. Liver was dissected out in ice-cold saline, thoroughly rinsed and homogenised to a known volume of certain buffer using a Potter-Elvejham homogeniser. Aliquots from the homogenate were used for further studies.

Glucose levels were measured using Glucometer Elite test according to Huistijn *et al*. (2001). Uric acid in serum was determined at 546 nm using enzymatic colorimetric method described by Fossati *et al*. (1980) and expressed in mg dl^{-1}. Serum total protein contents were determined using the Bio-Rad protein assay Reagent (Bio-Rad Laboratories, Hercules, CA. USA). Serum nitric oxide (NO) was determined by analysis of total nitrite using Griess reagent at 540 nm, 500 µM nitrate standard was used (Moshage *et al*., 1995). Serum samples were assayed for total antioxidant capacity (TAC) based on measuring the ability to hind the antioxidant of ox-brain homogenate as described by Stocks *et al*. (1974).

Tri-iodothyronine (T_3) and thyroxin (T_4) levels were measured in serum using an enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden) according to Chopra *et al*. (1971) and Liewendahi (1990), respectively. Also Serum insulin concentrations were estimated using an enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden) with rat insulin as standards (Starr *et al*., 1978).

Reduced glutathione (GSH) levels of the liver were determined by the method of Beutler *et al*. (1963). GSH in protein-free supernatant was determined at 412 nm and expressed in mg g^{-1} tissue. Liver lipid peroxide was measured by a colorimetric reaction with thiobarbituric acid-positive reactant substances (TBARS) and was expressed in terms of the malondialdehyde (MDA) concentration by using 1,1,3,3-tetraethoxypropane as a standard at 535 nm (Draper & Hadley, 1991). Catalase (CAT)

activity in homogenate liver tissue was determined according to the methods of Chance and Oshino (1973) in which the disappearance of peroxide is followed spectrophotometrically at 240 nm. One unit is defined as the amount of enzyme that decomposes one micromole of H_2O_2 per minute at 25°C and pH 7.0.

Statistical analysis

The results were presented as mean ± SE and significant differences were established using one way ANOVA test. All processing of data were conducted with the software packages Microsoft Excel XP for data storage and SPSS version 11.0 for statistical evaluation.

Results

The body weight of the all five groups was almost similar. It increased in the negative control group at the end of the 3 wks of the experiment as summarized in Fig. 1. On the other hand, positive control group significantly lost body weight as compared with negative control group. Body weights were significantly different in the melatonin-treated, EOH-extract treated and in combination-treatment groups from the positive control group at the end of the study (Fig. 2). However, there was a drastic increase in organ weight (organ wt / body wt ratio) including liver and testes in positive control group as compared with the treated groups (Fig. 2).

Induction of diabetes to male rats by a single dose of alloxan resulted a significant increase in serum glucose level of rats. The results also revealed a significant increase in serum uric acid level and liver lipid peroxidation product (MDA). These changes were accompanied with significant decreases in serum total protein content (Fig. 3), liver GSH level, liver catalase activity, serum NO as well as serum total antioxidant capacity of alloxan-induced diabetic rats (Table 1).

Serum total protein content of experimental animals did not show statistically significant differences between melatonin-treated ($p < 0.38$, $F_{1,19} = 1.4$) or EOH extract-treated ($p < 0.27$, $F_{1,19} = 0.86$) groups and diabetic group (Fig. 3). However, there was a significant increase ($p < 0.001$, $F_{1,19} = 22.5$) in the serum of the group treated with melatonin and EOH extract in combination when compared with the diabetic-induced rats.

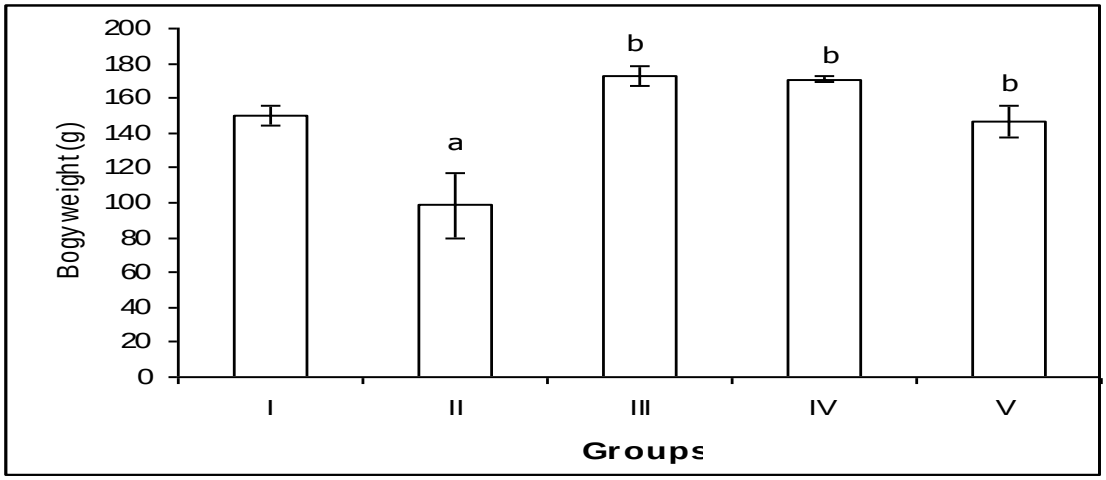

Fig. 1: Effect of melatonin and/or *C. droserifolia* treatment on body weight of diabetic rats. The values represent mean ± S.E. for each group (n = 10). [a]Significantly different from negative control group (I) (p< 0.01). [b]Significantly different from positive diabetic group (II) (p < 0.001). Group III, rats were treated with melatonin. Group (IV), rats were treated with EOH extract. Group (V), rats were treated with the melatonin and EOH extract in combination.

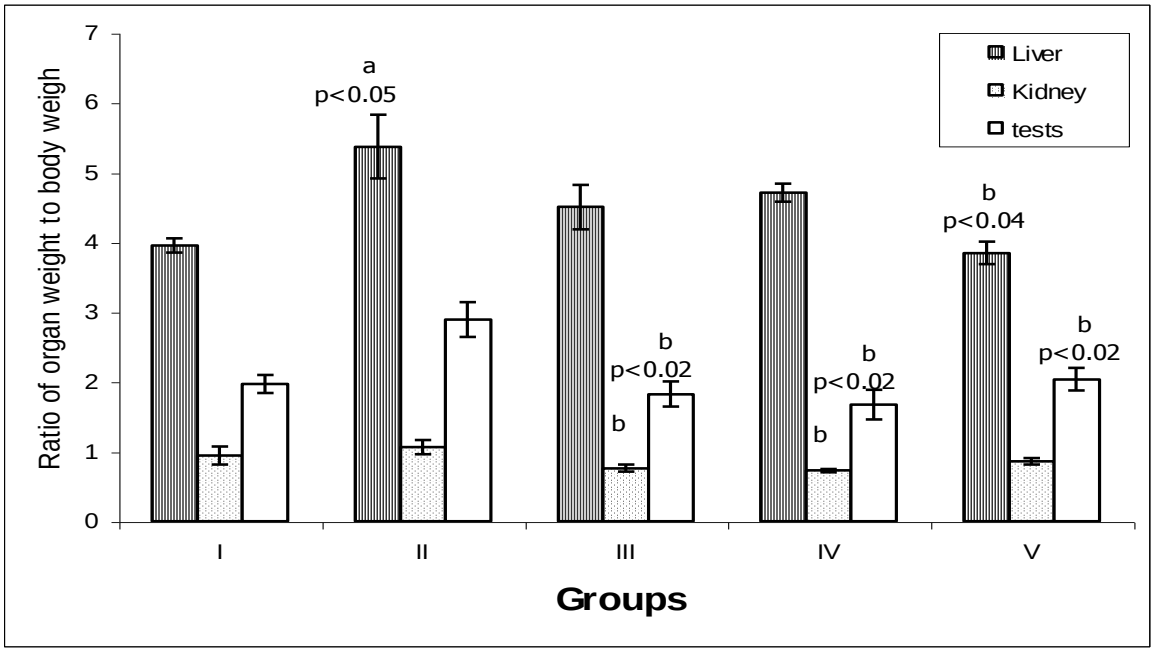

Fig. 2: Effect of melatonin and/or *C. droserifolia* treatment on the ratio of organ weight to body weight percentage in diabetic rats. The values represent the mean ± S.E. for ten rats for each group. [a]Significantly different from negative control group (I) for liver and tests (p< 0.05 and 0.02, respectively). [b]Significantly different from positive diabetic group (II) (p < 0.02-0.04). Groups III, IV and V as described in Fig 1.

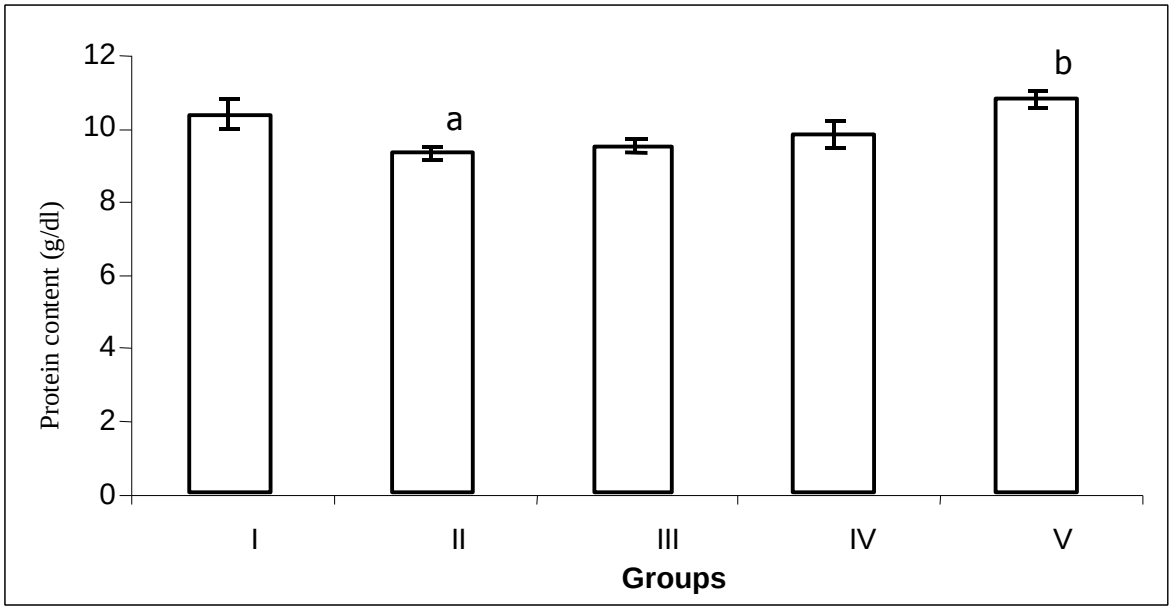

Fig. 3: Effect of melatonin and/or *C. droserifolia* treatment on serum total protein in alloxane-induced diabetic rats. The values represent the mean ± S.E. for each group (n = 10). [a]Significantly different from negative control group (I). [b]Significantly different from positive diabetic group (II). Groups III, IV and V as described in Fig 1.

By the end of the 3-wks period, blood glucose levels was significantly increased two times higher than controls ($p < 0.001$, $F_{1,19} = 64.2$) in alloxan-treated group (approximately 200 mg/dl). Melatonin was significantly reduced ($p < 0.001$, $F_{1,19} = 61.1$) the blood glucose levels as compared with control positive group. In the mean time, there was a significant difference between melatonin and EOH extract groups ($p < 0.01$) in their effect on blood glucose level separately or in combination (Table 1). Co-exposure of melatonin and EOH extract greatly enhanced the decrease in blood glucose (87.2 mg dl^{-1}) as compared with that of single exposure to extract (119.3 mg dl^{-1}).

NO level was significantly decreased by 63% in serum of diabetic group ($p < 0.001$, $F_{1,19} = 37.1$) as compared with negative control group. But its level increased significantly by 40.8% and 55.2% in the treated groups with EOH extract and melatonin ($p < 0.02$, $F_{1,19} = 7.9$ and $p < 0.002$, $F_{1,19} = 17.9$, respectively). However, the combination of both treatments inhibited the decreases of serum NO level in diabetic rats more significantly (57.7%) as compared with diabetic group ($p< 0.006$, $F_{1,19} = 11.5$). In the meantime, there was no significant difference between melatonin and EOH extract treatment ($p < 0.4$, $F_{1,19} = 0.6$).

There was also a significant increase ($p < 0.001$, $F_{1,19} = 26.86$) in the level of serum uric acid of positive control rats, as compared with negative controls (Table 1). Uric acid decreased significantly in diabetic rats, when treated with melatonin ($p < 0.03$, $F_{1,19} = 6.7$) or EOH extract ($p < 0.002$, $F_{1,19} = 17.1$), while the combination of both treatments did not affect the level of uric acid of the diabetic rats ($P< 0.57$, $F_{1,19} = 0.34$).

Evaluation of the degree of lipid peroxidation in liver was determined by measuring tissue MDA in nmol g^{-1} w.w after 3wks treatment. In the negative control group, the mean MDA was 62.88 ± 0.001 nmol g^{-1}. Liver MDA concentrations were significantly increased in diabetic animals (120.7 ± 11.6 nmol g^{-1}) as compared with negative control group ($p < 0.001$, $F_{1,19} = 36.25$). However, MDA level was significantly decreased in all treated groups ($p < 0.001$, $F_{1,19} = 30.8$-42.9) as compared with positive control rats. The group that was given melatonin did not show a statistically significant difference in MDA values as compared with that treated with EOH extract (Table 1).

GSH levels were significantly declined in liver of positive control as compared with those of negative control rats ($p < 0.001$, $F_{1,19} = 24.8$). Throughout the 3-wks study, the hepatic GSH levels were significantly elevated in the EOH extract- or melatonin treated groups ($p < 0.0001$, $F_{1,19} = 158.6$ and $p < 0.001$, $F_{1,19} = 44.26$, respectively) or in combined treatment ($p < 0.0001$, $F_{1,19} = 88.2$). Treatment with EOH extract or its combination with melatonin had more potent effect on GSH level than the group that treated with melatonin only (Table 1).
As seen in Table 1, there was a significant decrease in the CAT activity of diabetic rats ($p < 0.007$, $F_{1,19} = 12.6$) as compared with control . The activity was significantly increased in diabetic rats after treatment with melatonin or EOH extract ($p < 0.001$, $F_{1,19} = 43.7$) or with the combination of both treatments ($p < 0.001$, $F_{1,19} = 22.4$).

There was significantly difference between control and diabetic groups of serum TAC percentage ($p < 0.002$) on one hand and between diabetic and all treated groups on the other hand ($p < 0.004$). In the meanwhile, there was no any significantly difference between the different treatments (Table 1).

Table 1: Effect of melatonin and/or *C. droserifolia* extract treatments on selected biochemical parameters in serum and liver of alloxan–induced diabetic rats. I- negative control, II- positive control (diabetic), III- alloxan-induced and melatonin treatment (10 mg kg^{-1} day^{-1}), IV- alloxan-induced and *C. droserifolia* treatment (0.045 g kg^{-1} day^{-1}) and V- melatonin and *C. droserifolia* in combination.

Parameters	Groups I	II	III	IV	V
Serum					
Glucose (mg dl^{-1})	101.3 ± 8.3	199.8 ± 9.0 [a]	91.8 ± 7.1 [b]	119.3 ± 3.3 [b]	87.2 ± 4.9 [b]
NO (μm/ml)	110.7 ± 11.3	40.9 ± 2.1 [a]	63.5 ± 4.9 [b]	57.6 ± 5.5 [b]	64.5 ± 6.6 [b]
Uric acid (mg dl^{-1})	4.4 ± 0.05	5.0 ± 0.1 [a]	4.7 ± 0.07 [b,c,d]	4.4 ± 0.1 [b,c,d]	5.2 ± 0.2 [d]
TAC (%)	98.8 ± 0.5	95.3 ± 0.4 [a]	99.1± 0.5 [b]	99.0 ± 0.8 [b]	98.6 ± 0.8 [b]
Liver					
MDA (nmol g^{-1})	62.88 ± 0.001	120.7 ± 11.6 [a]	53.6 ± 3.5 [b]	53.6 ± 3.5 [b]	57.5 ± 2.4 [b]
GSH (mg g^{-1})	3.04 ± 0.03	2.7 ± 0.1 [a]	3.7 ± 0.1 [b]	3.5 ± 0.03 [b,d]	4.4 ± 0.04 [b,d]
CAT (U $X10^3$ g^{-1})	3.30 ± 0.40	1.3 ± 0.1 [a]	2.6 ± 0.1 [b,c]	2.1 ± 0.1 [b,c,d]	2.8 ± 0.20 [b,d]

Values are mean ± S.E (n =10). [a] Significantly different from negative control rats using one way ANOVA followed by Tukey's test. [b] Significantly different from positive diabetic rats. [c] Significantly different between the two treatments using paired samples test (SPSS) . [d] Significantly different between each single treatment and combination treatment using paired samples test (SPSS).

The levels of T_3, T_4, T_3/T_4 ratio and insulin are shown in table 2. Decreased serum T_3 ($p < 0.001$, $F_{1,19} = 32.9$) with slight altering serum T_4 ($p < 0.03$, $F_{1,19} = 7.1$) levels were observed in diabetic rats as compared with negative control group. Consequently, the T_3/T_4 ratio was significantly decreased in diabetic group. Significant increase in T_3 level was observed following treatment with each compound separately ($p < 0.001$, $F_{1,19} = 27.2\text{-}61.2$) or in combination ($p < 0.003$, $F_{1,19} = 17.8$). Serum T_4 level did not significantly change with EOH extract treatment or with its combination with melatonin, while it was highly significantly increased with melatonin treatment ($p < 0.0001$, $F_{1,19} = 54.04$). EOH extract and its combination with melatonin caused a more significant effect on T_3/T_4 ($p < 0.0001$, $F_{1,19} = 23.5$) than melatonin ($p < 0.03$, $F_{1,19} = 6.5$). Insulin was significantly decreased ($p < 0.0001$, $F_{1,19} = 34.2$) in diabetic rats and enhanced after the treatment with the EOH extract separately ($p < 0.02$, $F_{1,19} = 1.5$) or in combination with melatonin ($P < 0.01$, $F_{1,19} = 10.5$), while melatonin treatment did not affect the insulin level of diabetic group ($p < 0.4$).

Table 2: Effect of melatonin and/or *C. droserifolia* extract treatments on serum tri-iodothyronine (T_3), thyroxine (T_4), T_3/T_4 ratio and insulin levels of alloxan–induced diabetic rats. I, II, III, IV and V groups as described in the table 1.

Parameters	Groups				
	I	II	III	IV	V
T_3 (ng/dl)	358.5 ± 11.6 (321.3 -388.9)	279.7 ± 7.3 [a] (259.5 – 296.8)	338.1 ± 8.5 [b,c,d] (320.7 – 369.7)	436.0 ± 18.6 [b,c] (388.5 – 472.4)	442.3 ± 37.8 [b,d] (345.2 -526.4)
T_4 (µg/dl)	13.8 ± 0.4 (12.6 -14.4)	15.3 ± 0.4 [a] (14.4 – 17.0)	20.6 ± 0.5 [b,c,d] (18.8 – 21.7)	14.1 ± 0.3 [b,c] (13.3 – 14.9)	14.2 ± 0.3 [d] (13.4 – 14.7)
T_3/T_4 ratio	26.2 ± 1.4 (22.2 – 30.8)	18.3 ± 0.5 [a] (17.2 – 19.8)	16.5 ± 0.5 [b,c,d] (15.4 -18.1)	31.0 ± 1.2 [b,c] (28.1 – 35.3)	31.2 ± 2.6 [b,d] (24.3 – 36.4)
Insulin (µ U/ml)	42.5 ± 2.9 (31.4 – 48.1)	22.6 ± 1.6 [a] (17.6 – 26.6)	21.3 ± 0.8 [d] (20.0 - 24.4)	25.3 ± 1.3 [b,d] (20.9 – 29.3)	39.4 ± 5.0 [b,d] (29.3 – 53.7)

Values are mean ± S.E. (n =10). [a] Significantly different from respective normal control rats using one way ANOVA followed by Tukey's test. [b] Significantly different from diabetic rats. [c] Significantly different between the two treatments using paired samples test (SPSS). [d] Significantly different between each single treatment and combination treatment using paired samples test (SPSS) .

Examining correlations between diabetic rats and biochemical parameters using Spearman's and Pearson are presented in table 3. Diabetic rats had positive correlation only with MDA (0.55) and T_3 (+ 0.39) while they has negative correlation with other biochemical parameters. CAT had correlation with NO (+ 0.52) and LP (- 0.50). Moreover, TAC had positive correlation with GSH and CAT, while it had negative correlation with MDA.

Table 3: The relationships between diabetic and biochemical parameters of rats using Spearman's and Pearson correlation coefficients (r) to determine the levels of significance (p).

Parameter correlation	r	p	Parameter correlation	r	p
Glucose - GSH	-0.53	< 0.05	GSH - NO	-0.39	< 0.05
Glucose - MDA	0.55	< 0.01	MDA - CAT	-0.50	< 0.05
Glucose - TAC	-0.82	< 0.001	NO - CAT	0.52	< 0.01
Glucose - Uric acid	-0.58	< 0.01	TAC - GSH	0.47	< 0.05
Glucose - T3	0.39	< 0.01	TAC - MDA	-0.59	< 0.01
Glucose - T4	-0.36	< 0.05	TAC - CAT	0.46	< 0.05
T3 - T4	-0.89	< 0.001	NO - Uric acid	-0.52	< 0.01
GSH - MDA	-0.57	< 0.01			

Discussion

There is a clearly documented link between diabetic complications and the antioxidant system. Hyperglycemia causes a reduction in the levels of protective endogenous antioxidants and increases generation of free radicals. Most investigations of the response of diabetes mellitus to antioxidant substance have concerned on single compound and little is known of possible interactive effects of different antioxidant. As these latter seldom occur in nature, it is important to understand any interaction (synergistic or antagonistic) which may occur. Therefore, the present investigation dealt with important points concerning two aspects. The first one was the possible hypoglycemic activity of EOH extract of *C. droserifolia* on the oxidative stress of diabetic rats and compared with the effect of melatonin. The second aspect was the interaction between melatonin and EOH extract on diabetic rats.

Alloxan and streptozotocin are widely used to induce of diabetes mellitus in experimental animals and both agents cause selective destruction of pancreatic islet cells (Wilson Leiter, 1990). The possible mechanism for β-cells destruction by these chemicals was reported to include generation of some types of oxygen free radicals and alternation of endogenous scavengers of these reactive species (Ohkuwa *et al*., 1995). Pang *et al*. (1985) suggested that alloxan-induced diabetes might decrease pineal melatonin synthesis in rats by reducing the activity of hydroxyindole-o-methyltransferase, resulting in a decrease in pineal melatonin secretion.

Some defects have been shown in the antioxidant system in diabetes mellitus (McLennan *et al*., 1991). It has been suggested that ROS is a contributory factor in complications of diabetes mellitus (Soon Tan, 2002). There are many reports indicating the changes in parameters of oxidative stress in diabetes mellitus (Kakkar *et al*., 1998). Therefore, three kinds of oxidative stress markers were used to evaluate an oxidative stress; lipid peroxidation, plasma total antioxidant status and specific antioxidant defense systems. The results of the present study showed an increase in the level of MDA and a decrease in GSH content of the liver tissue of treated rat which are consistent with those results of Kakkar *et al*. (1998).

In diabetes, hypoinsulinaemia increases the activity of fatty acyl coenzyme A oxidase, which initiates β-oxidation of fatty acids, resulting in lipid peroxidation (Baynes, 1995). Also, protein glycation and glucose auto-oxidation can lead to the formation of free radicals, and this can induce lipid peroxidation (Baynes, 1991). Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (Baynes, 1995).

Three weeks post-treatment in the present investigation, the fasting glucose and hepatic MAD levels were significantly lower in the EOH extract- and/or melatonin-treated diabetic rats than in the positive control group. The EOH extract also significantly prevented the elevation of hepatic MDA formation. The hypoglycemic effect of *C. droserifolia* extract could be attributed to its flavonoid content (Winkelman 1989 and Yang *et al*., 1990). However, from the present data it can be suggested that the EOH extract may exert antioxidant activities that protect the tissues from the destructive damage of lipid peroxidation (Nicola *et al*., 1996).

Several findings supported the antioxidant activity of melatonin as it significantly inhibited MDA formation of rat liver in a concentration-dependent manner. The protection conferred by melatonin is likely due to its free radical scavenging ability in diabetic rats (Aksoy *et al*., 2003). However, there are several mechanisms by which melatonin may limit the generation of oxidative damage by reducing lipid peroxidation. It may acts directly as an effective scavenger of both the hydroxyl radical (Tan *et al*., 2000) and the peroxynitrite anion (Cuzzocrea *et al*., 1998), which are capable of initiating lipid peroxidation. Melatonin may also scavenge the peroxyl radical (Pieri *et al*., 1994, 1996)

which propagates the chain reaction of lipid breakdown. In addition, melatonin reportedly increases the activity of the antioxidative enzyme glutathione peroxidase (Barlow-Walden *et al*., 1995), inhibits the pro-oxidative enzyme nitric oxide synthase (Bettahi *et al*., 1996) and increases mRNA for antioxidant enzyme superoxide dismutase (SOD) in some tissues (Antolin *et al*., 1996).

From the present results, it was observed that the levels of liver GSH was lower in positive control rats as compared with negative controls. These results indicated that increased oxidative stress and accompanying decrease in antioxidants might be related to the causation of diabetes mellitus (Saxena, *et al*., 1993). The decreease in hepatic GSH could be a result of decreased synthesis or increased degradation of GSH by oxidative stress in diabetes. However, in the present study, a significant elevation of hepatic GSH level was observed in the extract-treated diabetic rats. This could indicate that the EOH extract can either increase the biosynthesis of GSH and/or reduce the oxidative stress leading to less degradation of GSH. On the other hand, the combined exposure to melatonin and EOH extract did not influence the hepatic GSH content more than those of single administration. Based on these findings, GSH enhancement may not be involved in the interaction between melatonin and EOH extract. In the meanwhile, melatonin or EOH extract treatment decreased MDA more than that of the combination exposure.

Uric acid content was increased significantly in all treated rat groups of the present study. It is suggested that uric acid acted together with GSH to offer a significant protection against oxygen free radical-induced liver injury (Sharma & Buetner 1993). It acted directly with various ROS and possessed antioxidant activity (Halliwell, 1994).

The data obtained from the present study revealed a significant reduction of hepatic CAT activity in positive control group as compared with negative control group. It is suggested that the reactive oxygen free radicals could inactivate and reduce the hepatic CAT activity. This observation is in agreement with Wohaieb *at al.* (1987). However, the hepatic CAT activity was significantly elevated in the EOH extract and/or melatonin-treated groups as compared with that of the positive control. CAT was involved in reducing ROS and improved the activities of the hepatic anti-oxidant enzymes (Saxena, *et al*., 1993 and Kakkar *et al*., 1998). The finding of the present work that melatonin had antihyperglycemic effect on diabetic rats is consistent with previous studies (Vural *et al*., 2001; Baydas *et al*., 2002 and Kamel *el al.*, 2002).

In the present investigation, it has been noted that NO significantly decreased in the positive diabetic rats as previously reported (Zhi-Shun *et al.*, 2001). The impairment of the nitric oxide has been suggested to be involved in autoimmune pancreatic β-cell destruction in insulin-dependent diabetes mellitus (Corbett & McDaniel, 1992 and Kolb & Kolb-Bachofen, 1992). Combined-treatment with melatonin and EOH crude extract was significantly increased NO level very similar to that of a single exposure to melatonin. These results indicate that the effect of concomitant melatonin with EOH crude extract-to enhance the NO level is greater than crude extract only. These results are in a disagreement with the finding of Fushiya *et al*. (1999) who reported that the suppression of NO production in activated macrophages *in vitro* are due to two flavonoids isolated as the active components from EOH extract of *C. droserifoli*a.

Most studies conducted in the two types of diabetic patients showed a significant decrease of the plasma total antioxidant status (Bonnefont-Rousselot *et al*., 2000). This status was lowered during an oral glucose tolerance test in normal and non-insulin dependent diabetic subjects, so that even an acute hyperglycemia can induce an oxidative stress (Bonnefont-Rousselot *et al*., 2000). However, the increase of the total antioxidant capacity of serum diabetic rats after treatment with melatonin and/or EOH extract could be attributed to absorption of antioxidants and

to improvement *in vivo* antioxidant status as described by Cao *et al*. (1998). Several human studies investigated a correlation between TAC and different individual antioxidants, such as, uric acid, albumin, vitamin A and E and bilirubin (Chapple 1997; Dailly *et al*. 1998; and Lands *et al*. 2000). Therefore, our study also focused on interrelation between TAC and other antioxidants. Moreover, TAC had positive correlation with GSH and CAT, whereas it had negative correlation with MDA. In experimentally induced diabetes in rats TAC significantly correlated with plasma albumin levels (Feillet-Coudray *et al*., 1999). Most investigators concluded that TAC appears to represent a mixed antioxidant response, rather than response to a single antioxidant. While being responsive to oxidative stress, the mechanisms of the response may differ between clinical situations, such that the clinical significance of changes in serum TAC remains to be defined.

Insulin deficiency in the diabetic state results in the impairment of glucose utilization leading to an increased generation of oxygen free radicals (Baynes, 1995). The present study revealed a very highly significant decrease in serum insulin level of diabetic rats and this finding agreed with the results of Sambandam *et al*. (2000) on STZ-induced diabetic rats. The dose of the EOH extract (0.045 g $kg^{-1}day^{-1}$) that was used in 3 wks study was found to be the optimum hypoglycemic dose. Single daily administration of the EOH extract for 21 days significantly reduced the serum glucose of diabetic rats and caused a significant change in the insulin levels. However, melatonin significantly decreased the glucose without causing any change in the serum insulin level and this means that the melatonin did not trigger insulin secretion for its glucose-lowering action. The present data demonstrated that the treatments of diabetic rats with EOH extract or its combination with melatonin caused marked ameliorations of hyperglycemia where the increase of insulin level became more pronounced after 3 wks of treatment. Improvement in insulin action in diabetic rats after EOH extract administration might be attributed to its ability to improve the physical state of plasma membrane through increment of hepatic reduced glutathione (GSH) levels thus interferes with the progression of lipid peroxidation. It appeared that, the effect of melatonin on insulin action could be synergistically enhanced by its combination with EOH extract.

Moreover, serum T_3 and T_4 levels were appropriate indicators of thyroid function (MacFarlane *et al*., 1984). In the present study, induction of diabetes with alloxan resulted in a decrease of serum T_3 levels. The decline in T_3 production resulted from decreased conversion of T_4 to T_3, whereas T_4 was unchanged. This observation could be attributed to the impaired microsomal conversion of T_4 to T_3 in damaged liver (Itoh *et al*., 1989). When insulin returned to its normal level, it was able to restore serum T_4 and T_3, hepatic conversion of T_4 to T_3, and T_3 production to normal levels. These observations were in agreement with that reported by Jennings (1984). Treatment of positive control rats with EOH extract or its combination with melatonin had more potent effect on insulin, T_3, T_4 and T_3/T_4 ratio than the treatment with melatonin only.

The major conclusions to be drawn from the present study are that the melatonin decreased response seen in diabetes by improving the glucose utilization processes; melatonin reduces the generation of oxygen free radicals in the body, resulting in lesser oxidative damage to the tissues, and enhanced levels of antioxidant potential activities in the diabetic rats. EOH extract not only exhibits hypoglycemic properties but also reduces oxidative stress in alloxan-induced diabetic rats and enhanced insulin release.

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مقارنة التأثير الوقائي للميلاتونين ومستخلص نبات السموة على موانع التأكسد فى الجرذان المصابة بمرض السكّر

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لقد أجريت الدراسة الحالية بهدف مقارنة تأثير الميلاتونين و مستخلص أوراق نبات السموه كلا على حدى أو معا على حالة موانع الأكسدة فى الجرذان المصابة بالسكّر. وقد أعطى الميلاتونين (10 ملجم/كجم) و مستخلص نبات السموه (0.045جم/كجم/ يوم) فى التجويف البروتونى للجرذان المصابة بمرض السكّر الناتج عن الحقن بمادة الألوكسان (110 ملجم/كجم). لقد أدت هذه المادة إلى ارتفاع نسبة مستوى السكر وإنخفّاض الأنسولين و إنخفاض مستويات هرمونات الغدة الدرقّية ونقص وزن جسم الحيوانات. هذه التغييرات كانت مصاحبة بالنقص المعنوىّ فى محتوى المصل من البروتينات ّ،و أكسيد النتريد، و القدرة الكليّة لمانع التأكسد، ومحتوى الكبد من الجلوتاثيون و إنزيم الكتاليز فى الجرذان المصابة بمرض السكّر. أثناء فترة الدراسة لم يؤثر الميلاتونين أو مستخلص نبات السموه كل منهما على حدى على مستوي البروتينات فى المصل بالمقارنة بالمجموعة الضابطة الموجبة ، بينما عاد إلى المستوى الطبيعي في حالة تعرّض الجرذان للمادتين معا. لقد زاد الميلاتونين مستويات ونشاطات علامات موانع التأكسد والتى تضمنت محتوى البروتين الكليّ، الجلوتاثيون , إنزيم الكتاليز و أكسيد النتريد بمقارن بالمجموعة الضابطة الموجبة. ولوحظ نقص مستوى malondialdehyde (MDA) في نسيج الكبد نقصا معنويا عند المعالجة بميلاتونين أومستخلص النبات. علاوة على ذلك، فإن معالجة الجرذان المصابة بمرض السكّر بالمستخلص المشار إليه بمفرده أو مجموعا مع الميلاتونين كان له تأثير أكثر فعالية على مستوى الجلوتاثيون فى الكبد،ومحتوى المصل من الأنسولين ،و هرمون ثلاثى يوديد الثيرونين (T_3) ، هرمون الثيروكسين (T_4) و نسبة T_3/ T_4 بالمقارنة بالمعالجة بمادة الميلاتونين فقط. وقد تبيّن من الدراسة الحالية بأن مستخلص نبات السموه كان أقل فعّالية على مستويات إنزم الكتاليز في الكبد بالمقارنة بتأثير الميلاتونين. كان لمحتوى التأكسد الكلى إرتباط إيجابي بمستوى الجلوتاثيون وإنزيم الكتاليز، بينما كان له إرتباط سلبي مع MDA. أكّدت النتائج بأنّ مرض السكّر زاد من حالة إجهاد الأكسدة في الكبد وأشارت إلى دور الميلاتونين في إخماد هذه الحالة عن طريق إزالت شقائق الأكسجين الحر وقدرته كمانع للتأكسد. وأيضا، قد تمكن مستخلص النبات المستخدم من منع التعقيدات المصاحبة لمرض السكّر بواسطة تقليل إجهاد الأكسدة وحماية الجرذان من ضرره. وبمقارنة تركيز كل من المادتين يتضح أن الميلاتونين بتركيزة الأقل أكثر فاعلية كمادة مضادة للأكسدة. وأوضحت النتائج أن التعرّض المصاحب للميلاتونين يزيد من تأثير المستخلص الذى استخدم كمادة معالجة لمرض السكّر فى الجرذان .