Zagazig J Pharm Sci., June, 2010
Vol. 19 No 1, pp 24-34

ISSN 1110-5089

POSSIBLE AMELIORATIVE ROLE OF SOME COMPOUNDS ON THE SIDE EFFECTS OF ROSIGLITAZONE

Mansour H. Zahra, Ahmed A. Hendawy and Reham Z. Hamza
Zoology Department, Faculty of Science, Zagazig University, Zagazig , Egypt.

ABSTRACT

The present study was carried out to evaluate the possible ameiolrative role of some compounds on the side effects of Rosiglitazone, *Nigella sativa*, Silymarin each alone and the combination of Rosiglitazone with either *Nigella sativa* or Silymarin. In order to get the best combination to avoid the possible side effects produced by Rosiglitazone. This was done through studying the effect of these plant extract and their combination on some lipid parameters and sex hormones ,seven groups of adult male rats each of 10 (200-250 gm) were used in this study. Hyperglycemia was induced in six groups of rats. Whereas, the 7th group was left as normal control group. All treatments were given orally daily for successive 28 days .The 1st group was left without treatment and kept as STZ diabetic. The 2nd group was administered Rosiglitazone (0.58mg/100gm) , The 3rd group was given *Nigella sativa*(0.25gm/100gm) , the 4th group was given Silymarin (50mg/100gm). The 5th and 6th groups were administered the combination of Rosiglitazone with either *Nigella sativa* or Silymarin respectively in the same recommended doses. Blood samples were collected after 1st, 2nd, 3rd and 4th week post drug administration. Serum was separated and used for determination of various variables. The results showed that Rosiglitazone afforded a marked decrease in serum Triglycerides, Total cholesterol, LDL-c, vLDL-c levels as well as a slight to significant increase in serum HDL concentration along the course of the study and decreased testosterone hormone. Treatment of diabetic rats with various treatments elicited a marked decrease in serum Triglycerides, Total cholesterol, LDL-c, vLDL-c as well as a marked increase in serum HDL-c level when compared with diabetic non treated group and diabetic group treated with Rosiglitazone drug, the histopathological changes were also studied.

INTRODUCTION

Diabetes was described more than 2000 years ago .For the past 200 years, it has features in the history of modern medicine .Since the discovery of insulin, work on diabetes at the cellular and clinical levels has expanded as fast as new laboratory and diagnostic technique allow [1].

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and /or insulin action [2].

Diabetes mellitus is associated with very subtle disorders, affects either directly or indirectly, various functions as the reproductive system. Sexual dysfunction in all its forms (reduced erection, impotence, and other libido dissociations) is an accompanying phenomenon of the diabetic disease .These disorders are related to the regulation of carbohydrates metabolism and to the duration of disease, they are not necessary correlated with sexual dysfunction [3].

The WHO expert committee on diabetes mellitus recommendations of 1980[4] included investigation of hypoglycemic agents from plants used in traditional medicine. *Nigella sativa* oil have been used for treatment of experimentally induced diabetes in animals based on its, combined hypoglycemic and immunopotentiating effects that help in ameliorating the impaired immunity and infections associated with diabetes [5, 6].

A whole range of pharmacological agents are available to ameliorate the T2DM symptoms by different mechanisms. A reduction in insulin resistance at any stage of T2DM will improve glucose metabolism by allowing the endogenous insulin to be more effective. The use of different insulin sensitizers and secretagogues, either in single therapy or in combination, would help to improve hyperglycemia , either by increasing peripheral glucose uptake, improving insulin secretion, decreasing hepatic glucose output or reducing the influx of glucose to the body[7].

Rosiglitazone came under heavy security after 21 May 2007, when the NEjM published online a meta analysis of other studies into the drug's efficacy and safety .The results showed that the drug increased the risk of heart attack by 43 %in people who took it for at least 24 weeks [8].

Rosiglitazone manufactured by Glaxo Smithkline (GSK), was approved as an adjunct to diet and exercise to improve control of blood sugar levels .Rosiglitazone

is approved to be used as a single therapy or used in combination with metformin and sulfonylurea, or with other oral anti-diabetes treatments [9]. In the third quarter of 2007, Sales of Rosiglitazone were down 38 % from a year earlier world wide and down 48 % in the United States [10].

A number of natural products exhibit properties that could be used as remedies to improve glucose metabolism [11] some plants extracts can significantly reduce blood glucose levels and lipids, improving insulin sensitivity [12].

Nigella sativa has a great potential in the treatment of diabetic animal because of its combined hypoglycemic[13] and immunopotentiating properties, hypotensive [14], hepatoprotective , it is cheap and readily available . M iny studies have also examined the antidiabetic effect of *Nigella sativa*.

Traditional antidiabetic plants provide useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to existing therapies. A scientific investigation of traditional herbal remedies for diabetes mellitus may provide valuable leads for the development of alternative drugs and therapeutic strategies alternative are clearly needed because of the inability of current therapies for many rural populations, particularly in developing countries [15].

Silymarin has been used for more than 2000 years as a natural remedy for treating hepatitis and cirrhosis and to protect liver from toxic substances . Silymarin acts by anti-oxidative, anti-lipid peroxidative, antifibrotic, and anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanisms in experimental liver diseases .Furthermore, Silymarin has been extensively studied, both *in vivo* and *in vitro*, as chemopreventive agent against various cancers [16].

Therefore, the study aimed to give an insight about the possible role of two natural products (N. *sativa* seeds and silymarin) on the risk factors of Rosiglitazone on the cardiovascular system. Moreover, it will put pits on pieces about it's role on sexual dysfunction caused by diabetes.

EXPERIMENTAL

This study was carried out on 70 mature male albino rats weighing 200-250 gm each. They were divided into 7 equal groups (each of 10) as follows :-

Induction of diabetes:

After induction of diabetes by injecting rats with STZ I.P in a dose of 50 mg/kg, rats with fasting blood glucose level more than 250mg/dl were considered diabetic.

I- The 1st group (STZ group)

Animals were served as diabetic non treated group for other diabetic groups.

II- The 2nd group (STZ + Rosiglitazone treated group)

Animals were given a daily oral dose of AVA (0.58 mg/100g.b.wt) dissolved in 1 ml of 1% Tragacanth gum as suspension for four weeks.

III- The 3rd group (STZ+ *Nigella sativa* extract treated group)

Animals were received a daily oral dose of *Nigella sativa* extract (0.25gm/100g b.wt) for four weeks.

VI- The 4th group (STZ+ Silymarin extract treated group)

Animals were given daily dose of Silymarin extract (50mg/kg.b.wt) suspended in 1 ml of 1 % CMC suspension orally for four weeks daily.

V- The 5th group (STZ + AVA + *Nigella sativa* extract treated group)

Animals were received a daily oral dose of AVA (0.58mg/100g b.wt) as previously mentioned combined with *Nigella sativa* extract (0.25gm/100 b.wt), orally for 4 weeks.

VI- The 6th group (STZ + AVA + Silymarin extract treated group)

Animals were received a daily oral dose of AVA (0.58mg/100g, b.wt) as prepared as mentioned above with a daily dose of Silymarin extract (50mg/kg.b.wt) orally for four weeks.

VII- The 7th group (control group)

Animals were served as normal control group given 1ml citrate buffer (PH=4.5) (The vehicle in which STZ was dissolved) daily orally for 4 weeks.

ISSN 1110-5089

Blood sampling:

After the end of the experiment, blood samples were collected after the end of 1st, 2nd, 3rd and 4th week post drugs administration from the retro orbital plexus using microhaematocrit capillary tubes into centrifuge tubes. Serum was harvested from blood without anticoagulant and used for determination of serum Triglycerides [17], Total cholesterol [18], LDL-c, HDL-c, vLDL-c [19], F.S.H L.H and testosterone [20].

After 4 weeks post drug administration, animals were sacrificed and a sample from testis was fixed in 10% formalin for histopathological studies [21]

Statistical analysis:

Data were collected and analyzed using the computer program SPSS / Pc+ (2001). The statistical method used was one way ANOVA test (F-Test) according to [22]

RESULTS

The results of the experiment revealed the following observations

(1) Effect on some lipid parameters:

(A) Effect on serum Triglycerides:-

Table (1) revealed that treatment of rats with STZ induced a significant increase in serum triglycerides level along the course of the study when compared with control group

Meanwhile Rosiglitazone, Silymarin, *N.sativa* and their continuations for 28 days to diabetic rats significantly decreased triglycerides level along the course of the entire period of the experiment when compared with STZ treated group (table 1)

(B) Effect on serum total cholesterol:-

STZ diabetic rats showed a significant increase along the entire period of the study compared with buffer group. Treatment of diabetic rats with Rosiglitazone, N.sativa, Silymarin and their continuations for 28 days exhibited a significant decrease ($P < 0.05$) in serum total cholesterol when compared with STZ diabetic group along the course of the study (table 2).

I Effect on serum High density lipoprotein Cholesterol; (HDL-c):-

The STZ diabetic rats showed a non-significant change in HDL-c level along the course of the experiment when compared with buffer groups

Rosiglitazone elicited a significant elevation in serum HDL-c of STZ diabetic rats after 1st week post treatment together with a slight increase after 2nd, 3rd and 4th week of the study compared with buffer group. Whereas other treatment elicited non-significant changes in serum HDL-c of STZ diabetic rats along the course of the experiment except group treated with *Rosiglitazone + N sativa* and Rosiglitazone + Silymarin which showed a significant decrease in HDL-c after 3rd and 4th weeks post-drugs administration when compared with STZ diabetic group (table 3).

(D) Effect on serum Low density lipoprotein (LDL-C):

Concerning the effect of various treatments on serum LDL-c of diabetic rats, the obtained results showed that STZ afforded a marked increase along the entire course of the study when compared with buffer group.

Treatment of STZ diabetic rats with Rosiglitazone, Silymarin, N.sativa and their continuations for 28 days elicited a significant decrease in serum LDL-c level along the entire period of the experiment when compared with STZ diabetic rats. (table 4)

(E) Effect on serum very Low density lipoprotein (vLDL-c):

The results revealed that STZ induced a significant elevation in serum vLDL-c along the entire period of the experiment when compared with buffer group. On the contrary, Rosiglitazone, Silymarin, N.sativa and their continuations afforded a significant decrease in serum vLDL-c of diabetic rats along the entire period of the study. (table 5).

(2) Effect on some sex hormones:

(A) Effect on serum Testosterone hormone:

The testosterone level of the STZ treated group revealed a marked decrease along the entire period of the experiment (4 weeks) when compared with buffer treated group

Serum testosterone level of the diabetic group treated with *N. sativa* for successive 28 days revealed a marked elevation when compared with either STZ or buffer treated groups along the course of the experiment.

Treatment of STZ treated group with the recommended dose of Rosiglitazone for 4 weeks elicited a

significant decrease in serum testosterone level ($P < 0.05$) along the entire period of the experiment when compared with STZ non-treated group. While diabetic groups treated with silymarin or Rosiglitazone + *N. sativa* or Rosiglitazone + silymarin showed non-significant changes when compared with STZ treated group (table 6).

(B) Effect on serum Follicle Stimulating hormone (F.S.H):

STZ treated group showed a marked decrease in serum F.S.H. level when compared with control group along the course of the experiment (table 7).

The same decrease was reported in diabetic rats treated with either silymarin or *N. sativa* along the entire period of the experiment except after the first week for the group treated with *N. sativa* which showed a non-significant decrease, a marked increase in FSH level was obtained along the course of the experiment in the group treated with Rosiglitazone compared with STZ diabetic rats.

(C) Effect on serum Leutnizing hormone (L.H):

Concerning the effect of different treatments on serum L.H. level of diabetic rats, the obtained results revealed that STZ treated group showed a significant decrease when compared with control group along the entire course of the experiment.

Whereas, Rosiglitazone or combination of Rosiglitazone with either *N.sativa* or silymarin induced a significant elevation in serum L.H level along the course of the experiment when compared with buffer and STZ diabetic group. Unlike diabetic treated groups with silymarin or *N. sativa* which showed a significant decrease in serum L.H. level along the course of the study when compared the STZ treated group (table 8).

Table (1): Effect of Rosiglitazone drug, *Nigella sativa*, Silymarin and their combinations on serum triglycerides concentration (mg/dl) in diabetic male albino rats (mean ± SE). (N = 7).

Groups	Triglycerides (1st Week)	Triglycerides (2nd Week)	Triglycerides (3rd Week)	Triglycerides (4th Week)
1. STZ (diabetic non treated group)	98.16±2.22^{a}	100.50±2.33^{a}	105.00±0.42^{a}	84.60±0.42^{a}
2. STZ + Rosiglitazone treated group	85.00±1.80^{b}	81.46±3.61^{bc}	82.00±1.67^{b}	87.16±1.35^{a}
3.STZ +Silymarin treated group	74.38±3.05^{c}	69.06±3.70^{cd}	61.91±2.85^{c}	60.58±2.56^{c}
4. STZ +*Nigella sativa* treated group	71.33±3.44^{c}	67.50±2.27^{cd}	64.83±3.83^{c}	57.50±3.27^{c}
5. STZ + Rosiglitazone + *Nigella sativa* treated group	69.91±5.97^{cd}	60.58±3.33^{d}	52.16±2.44^{d}	52.50±2.75^{cd}
6. STZ+Rosiglitazone+Silymarin treated group	69.83±2.70^{cd}	67.78±1.61^{d}	60.38±1.31^{d}	62.33±1.22^{c}
7.Control group	73.33±4.52^{c}	72.16±4.02^{cd}	68.33±6.08^{b}	72.66±3.86^{bc}

Means within the same column in each category carrying different letters are significant at ($P \leq 0.05$).

Table (2): Effect of Rosiglitazone drug, *Nigella sativa*, silymarin and their combinations on serum cholesterol concentration (mg/dl) in diabetic male albino rats (mean ± SE). (N = 7).

Groups	Cholesterol (1st Week)	Cholesterol (2nd Week)	Cholesterol (3rd Week)	Cholesterol (4th Week)
1. STZ (diabetic non treated group)	134.83±4.82^{a}	147.50±5.56^{a}	156.05±4.03^{a}	171.00±3.16^{a}
2. STZ + Rosiglitazone Group	84.16±2.62^{b}	82.50±2.41^{b}	66.50±1.52^{b}	61.16±1.38^{c}
3. STZ + Silymarin Group	52.08±1.34^{e}	48.66±1.37^{cd}	36.33±1.45^{d}	27.50±2.14^{de}
4. STZ + *Nigella sativa* Group	81.83±3.20^{b}	80.08±1.93^{b}	78.16±2.26^{b}	71.08±2.93^{b}
5. STZ + Rosiglitazone + *Nigella sativa* Group	66.16±2.43^{c}	58.00±3.76^{c}	47.50±2.48^{cd}	36.66±2.24^{d}
6. STZ + Rosiglitazone + Silymarin Group	62.33±2.02^{cd}	56.08±3.27^{c}	57.85±1.54^{c}	54.66±1.98^{cd}
7.Control group	57.83±4.48^{de}	57.83±6.75^{c}	56.66±6.13^{c}	54.50±3.84^{cd}

Means within the same column in each category carrying different letters are significant at ($P < 0.05$).

ISSN 1110-5089

Zagazig J. Pharm. Sci., June, 2010
Vol. 19, No. 1, pp. 24-34

Table (3): Effect of Rosiglitazone drug, *Nigella sativa*, *Silymarin* and their combinations on serum high density lipoprotein (HDL) concentration (g/dl) in diabetic male albino rats (mean ± SE). (N = 7).

Groups	HDL (1st Week)	HDL (2nd Week)	HDL (3rd Week)	HDL (4th Week)
1. STZ (diabetic non treated group)	34.83±1.44^{b}	36.66±0.49^{ab}	41.50±1.11^{a}	39.00±0.96^{ab}
2. STZ + Rosiglitazone Group	43.50±3.28^{a}	39.83±1.19^{a}	42.33±1.17^{a}	41.66±0.66^{a}
3. STZ + Silymarin Group	36.50±0.56^{b}	33.50±1.23^{b}	34.50±0.92^{bc}	30.33±1.17^{d}
4. STZ + *Nigella sativa* Group	38.00±1.03^{b}	35.50±1.74^{b}	37.16±1.24^{ab}	35.50±1.74^{b}
5. STZ + Rosiglitazone + *Nigella sativa* Group	39.33±0.88^{b}	34.50±1.33^{b}	35.83±1.32^{bc}	34.16±1.07^{c}
6. STZ + Rosiglitazone + Silymarin Group	39.16±1.01^{b}	33.16±1.19^{b}	32.50±0.42^{c}	31.33±0.49^{cd}
7.Control group	34.34±1.52^{b}	35.66±1.80^{b}	36.33±0.49^{b}	36.66±0.91^{b}

Means within the same column in each category carrying different letters are significant at ($P \leq 0.05$).

Table (4): Effect of Rosiglitazone drug, *Nigella sativa*, *Silymarin* and their combination on serum low density lipoprotein (LDL) concentration (g/dl) in diabetic male albino rats (mean ± SE). (N = 7).

Groups	LDL (1st Week)	LDL (2nd Week)	LDL (3rd Week)	LDL (4th Week)
1. STZ (diabetic non treated group)	85.46±8.41^{a}	91.37±9.04^{a}	92.16±3.91^{a}	111.90±5.64^{a}
2. STZ +Rosiglitazone Group	28.83±2.65c	28.13±3.44^{c}	20.53±2.38^{c}	19.52±2.43^{c}
3. STZ +Silymarin Group	8.23±0.98d	7.22±1.13^{d}	8.06±0.80^{d}	7.51±1.62^{e}
4. STZ + *Nigella sativa* Group	48.06±2.31^{b}	44.08±3.71^{b}	31.63±3.93^{b}	44.08±3.71^{b}
5. STZ + Rosiglitazone + *Nigella sativa* Group	32.61±2.72^{bc}	24.71±3.04^{c}	5.30±0.58^{e}	13.00±1.74^{d}
6. STZ +Rosiglitazone+ Silymarin Group	23.97±1.20^{c}	18.02±2.38^{cd}	18.18±2.78^{c}	16.86±2.89^{cd}
7.Control group	21.60±3.65c	21.60±3.37c	19.66±1.44c	20.26±2.88c

Means within the same column in each category carrying different letters are significant at ($P \leq 0.05$).

Table (5): Effect of Rosiglitazone drug, *Nigella sativa*, *Silymarin* and their combinations on serum very low density lipoprotein (VLDL) concentration (g/dl) of diabetic male albino rats(mean ± SE). (N = 7).

Groups	VLDL (1stWeek)	VLDL (2nd Week)	VLDL (3rd Week)	VLDL (4th Week)
1. STZ (diabetic non treated group)	44.33±2.40^{a}	45.00±2.62^{a}	47.80±2.21^{a}	48.109±3.22^{a}
2. STZ +Rosiglitazone Group	15.00±0.36^{b}	16.29±0.32^{b}	17.95±0.44^{b}	17.43±0.27^{b}
3. STZ + Silymarin Group	12.87±1.21^{b}	11.81±1.14^{c}	4.38±0.57^{d}	2.31±0.71^{e}
4. STZ +*Nigella sativa* Group	12.26±1.28^{bc}	11.50±1.25^{bc}	9.36±1.36^{c}	11.50±1.25^{c}
5. STZ+ Rosiglitazone + *Nigella sativa* Group	11.98±1.19^{bc}	10.11±1.06^{cd}	6.43±0.68^{c}	5.50±0.55^{d}
6. STZ + Rosiglitazone +Silymarin Group	11.96±0.54^{c}	11.55±0.52^{c}	6.87±0.22^{c}	6.46±0.24^{cd}
7.Control group	14.95±2.08^{b}	14.43±2.20^{bc}	15.66±2.21^{b}	15.20±2.66^{b}

Means within the same column in each category carrying different letters are significant at ($P \leq 0.05$).

Table (6): Effect of Rosiglitazone drug, *Nigella sativa*, *Silymarin* and their combinations on serum total testosterone hormone (µIU/ml) of diabetic male albino rats (mean ± SE). (N = 7).

Groups	testosterone (1stWeek)	testosterone (2nd Week)	testosterone (3rd Week)	testosterone (4th Week)
1. STZ (diabetic non treated group)	1.95±0.33^{cd}	2.15±0.27^{cd}	2.22±0.24^{c}	2.27±0.24^{c}
2. STZ + Rosiglitazone Group	0.58±0.11^{e}	0.89±0.11^{e}	0.70±0.11^{d}	0.67±0.13^{d}
3. STZ + Silymarin Group	2.55±0.64^{bc}	2.86±0.58^{c}	3.04±0.64^{c}	3.13±0.65^{c}
4. STZ + *Nigella sativa* Group	8.12±1.10^{a}	8.71±1.14^{a}	9.14±1.14^{a}	9.50±1.07^{a}
5. STZ + Rosiglitazone + *Nigella sativa* Group	2.56±0.55^{bc}	2.80±0.50^{c}	2.99±0.44^{c}	3.12±0.42^{c}
6. STZ + Rosiglitazone + Silymarin Group	1.77±0.38^{cd}	1.97±0.45^{cd}	2.37±0.39^{c}	2.68±0.35^{c}
7.Control group	4.77±0.51^{b}	5.12±0.45^{b}	5.53±0.37^{b}	5.73±0.35^{b}

Means within the same column in each category carrying different letters are significant at ($P \leq 0.05$).

Table 7: Effect of Rosiglitazone drug, *Nigella sativa*, *Silymarin* and their combinations on serum follicle stimulating hormone (F.S.H) (µIU/ml) of diabetic male albino rats (mean ± SE). (N = 7).

Groups	F.S.H (1st Week)	F.S.H (2nd Week)	F.S.H (3rd Week)	F.S.H (4th Week)
1. STZ (diabetic non treated group)	0.69±0.20^{c}	0.66±0.20^{c}	0.61±0.19^{c}	0.58±0.18^{c}
2. STZ + Rosiglitazone Group	2.15±0.42^{a}	2.68±0.51^{a}	3.14±0.47^{a}	3.40±0.47^{a}
3. STZ + Silymarin Group	0.21±0.01^{c}	0.19±0.01^{d}	0.17±0.08^{d}	0.14±0.01^{c}
4. STZ+ *Nigella sativa* Group	0.34±0.03^{dc}	0.30±0.04^{d}	0.27±0.04^{d}	0.25±0.04^{d}
5. STZ + Rosiglitazone + *Nigella sativa* Group	0.75±0.17^{c}	0.70±0.16^{c}	0.65±0.15^{c}	0.45±0.16^{cd}
6. STZ +Rosiglitazone + Silymarin Group	0.46±0.14^{d}	0.43±0.15^{cd}	0.39±0.15^{cd}	0.35±0.15^{cd}
7.Control group	1.26±0.19^{b}	1.21±0.20^{b}	1.07±0.17^{b}	1.02±0.17^{b}

Means within the same column in each category carrying different letters are significant at (P ≤ 0.05).

Table 8: Effect of Rosiglitazone drug, *Nigella sativa*, *Silymarin* and their combinations on serum luteinizing hormone (LH) (µIU/ml) of diabetic male albino rats (mean ± SE). (N = 7).

Groups	LH (1st Week)	LH (2nd Week)	LH (3rd Week)	LH (4th Week)
1. STZ (diabetic non treated group)	0.43±0.04^{c}	0.32±0.03^{d}	0.20±0.03^{d}	0.13±0.04^{d}
2. STZ + Rosiglitazone Group	2.76±0.54^{a}	2.87±0.52^{a}	2.95±0.50^{a}	3.05±0.48^{a}
3. STZ + Silymarin Group	0.30±0.02^{d}	0.26±0.03^{d}	0.23±0.04^{d}	0.18±0.05^{d}
4. STZ +*Nigella sativa* Group	0.30±0.02^{d}	0.27±0.01^{d}	0.20±0.03^{d}	0.18±0.02^{d}
5. STZ +Rosiglitazone +*Nigella sativa* Group	1.04±0.11^{b}	0.99±0.09^{b}	0.91±0.08^{b}	0.82±0.06^{b}
6. STZ + Rosiglitazone +Silymarin Group	1.70±0.37^{ab}	1.61±0.37^{ab}	1.52±0.35^{ab}	1.42±0.37^{ab}
7.Control group	0.41±0.06^{cd}	0.45±0.05^{cd}	0.39±0.05^{cd}	0.38±0.06^{c}

Means within the same column in each category carrying different letters are significant at (P ≤ 0.05)

1- Rosiglitazone treated group :

Microscopically , the testes of the male Rosiglitazone treated rats were edematous in some cases; in other cases the testis appears oval in size (.Fig 1). Marked atrophic seminiferous tubules lined by few layers of spermatogenic cells with absence of sperms as shown in (Fig. 2).

2- Rosiglitazone+*Nigella sativa* :

Grossly and microscopically, the testes were normal with normal spermatogenesis (Fig3)

3- Rosiglitazone + Silymarin:

Slight edema of the testicular tissues was seen microscopically with normal somniferous tubules (Fig . 4)

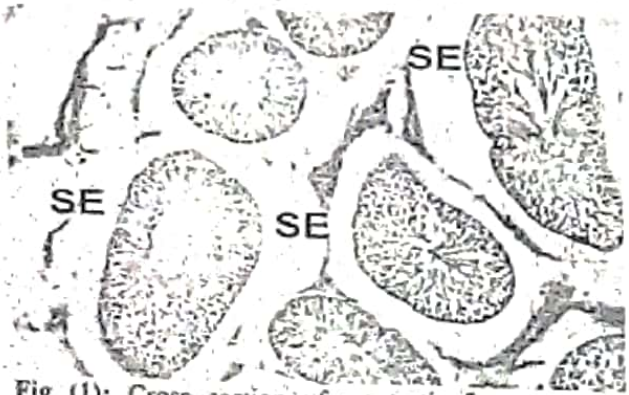

Fig (1): Cross section of rat testis from group A showing marked severe edema .H & E X (150) (SE : Severe Edema).

Fig (2): Cross section of rat testis from group A showing congestion of blood vessels of tunica albugina with azospermia. H&EX(150) (As:Azospermia) .

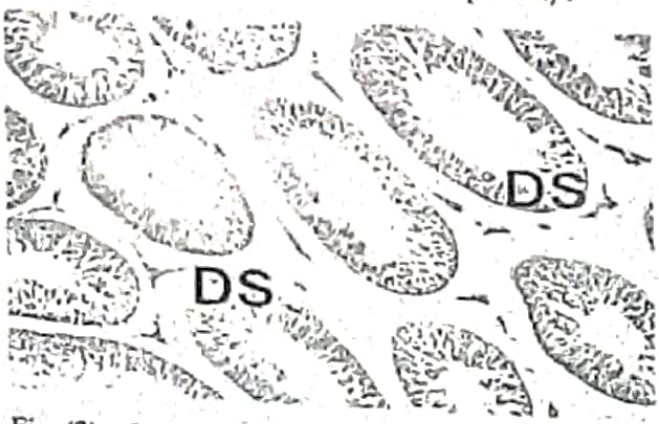

Fig (3): Cross section of rat testis from group B-I Showing normal testis .H & E Stain X (150).

Zagazig J. Pharm. Sci., June, 2010
Vol. 19, No. 1, pp. 24-34

ISSN 1110-5089

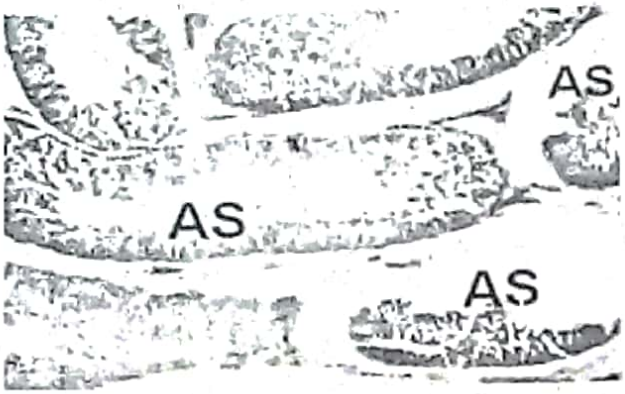

Fig (4): Cross section of rat testis from group C-I Slight edema of the testicular tissues were seen microscopically with normal seminiferous tubules. (NTT: Normal Testicular Tissue)

DISCUSSION

The present study was an attempt to evaluate the hypoglycaemic effect of Rosiglitazone, *Nigella sativa*, silymarin each alone and the combination of Rosiglitazone with either *N. sativa* or silymarin when given to normal and diabetic rats for 28 successive days on some lipid parameters (triglyceride, total cholesterol, LDL-c, HDL-c and VLDL-c) were also studied. Some sex hormones (serum F.S.H, L.H, and testosterone).

Because of low cost, traditional medicinal plants also raise significant interest to prevent morbidity and mortality from chronic diseases in low and middle income populations [15].

Effect on lipid parameters:

Our results revealed that Rosiglitazone, *N. sativa*, silymarin and their combination when given daily for 28 successive days afforded significant decrease in serum triglyceride along the entire period of the experiment in hyperglycemic rats when compared with STZ group (table 1).

Whereas, STZ- treated group showed a marked elevation in serum triglycerides when compared with buffer group along the entire course of the study. Meanwhile, various treatments elicited a marked decrease in serum triglycerides along the course of the study when compared with STZ diabetic group. These values were reverted to the buffer value after the 3rd and 4th weeks post-treatment in the groups given silymarin + Rosiglitazone, whereas, a marked decrease were reported in the same periods in the groups given silymarin alone and Rosiglitazone + *N. sativa*.

Our results showed decrease triglycerides, total cholesterol, LDL and vLDL-c (tables 1,2,4,5) with the various treatments, pari passu with the results of [16]. They reported that administration of silymarin to high Cholesterol fed rats afforded a significant decrease in serum levels of total lipids, triglycerides, total cholesterol, LDL-c and VLDL-c as well as HDL-C.

On serum total cholesterol, the obtained results showed non-significant changes except the group given silymarin and /or its combination with Rosiglitazone after the 3rd week, and the group given *N. sativa* after the 4th week which showed a marked decrease when compared with normal control group. Whereas, STZ-treated group revealed a marked elevation in serum total cholesterol along the entire period of the study when compared with buffer group. Treatment of all diabetic groups with various plant drugs for 28 successive days afforded a marked decrease in serum total cholesterol when compared with STZ – treated group along the entire course of the study. However, treatment of diabetic group with Rosiglitazone, elicited a marked decrease in serum total cholesterol when compared with buffer group at 3rd and 4th week post-treatment together with a marked decrease than buffer value after 3rd and 4th week post-treatment of diabetic group. The same previous response was recorded in the group given combination of Rosiglitazone with *N. sativa*. Whereas, the combination of Rosiglitazone + silymarin reverted the cholesterol value to nearly its normal value after the 3rd and 4th week post-treatment .Induction of diabetes with STZ exhibited non-significant. Changes in HDL-c along the entire period of the experiment when compared with buffer group . Treatment of STZ – diabetic group with Rosiglitazone afforded a significant elevation in serum HDL-c after the 1st week and a nonsignificant increase after 2nd , 3rd and 4th weeks post drug administration when compared with STZ-diabetic group . Treatment of diabetic groups with either silymarin , *N. sativa* and their combination with Rosiglitazone elicited nonsignificant changes in serum HDL-c in the treated groups when compared with STZ-diabetic group except *N. sativa* treated group which showed a nonsignificant change as well as the group treated with Rosiglitazone + N.sativa after 4th week , In STZ diabetic group the LDL-c values showed a marked increase when compared with buffer group along the entire course of the study. Treatment with various drugs caused a marked decrease in LDL-c values when compared with STZ-diabetic group a long the entire period of the experiment. Treatment of STZ- diabetic group with Rosiglitazone reverted the LDL-c values to nearly their buffer values after the first two weeks, whereas, silymarin reverted values to nearly their normal values along the last 3 weeks of the study

together with a significant decrease after the 1st week when compared with buffer group. Silymarin treatment of STZ diabetic group afforded a marked decrease in serum LDL-c along the entire period of the study whereas, combinations of Rosiglitazone with either *N. sativa* or silymarin elicited a marked decrease in serum LDL-c of diabetic groups along the entire period of the study when compared with buffer group except after the 3rd week post- treatment with Rosiglitazone + Silymarin.

More recently, [23] it has been reported that N.sativa seed have a significant lowering effect on total cholesterol level and LDL cholesterol level.

[24] reported that since *N. sativa* (Kalonji) reduced the total cholesterol level, there is a probable decrease in intracellular cholesterol level which cause an up-regulation of LDL – receptor.

Their results suggest that *N. sativa* has a protective role in atherosclerosis due to its hypolipidemic activity. These authors added that treatment of rats with *N sativa* petroleum extract for 4 weeks afforded lowering of triglycerides and increased HDL-echolesterol. Nearly simular results were previously reported by [25]. They studied thymoquinone (active ingredient of *N sativa* seeds) on Doxorubicin-induced hyperlipidemic nephropathy in rats. They found that thymoquinone afforded a significant lowering of triglycerides and total cholesterol.

Our results are in agreement with [26] they reported that when *N. sativa* was administered in a dose of 800mg/kg of rats for 4 weeks elicited a significant decrease in serum total cholesterol, LDL-c, triglycerides and a significant elevation in serum HDL-c level.

Our results were compatible also with [27] they reported that silymarin induced a decrease of plasma cholesterol, LDL-c, VLDL-c and increase in HDL-c. These changes are considered to be of benefit in pharmacological treatment of hypercholesterolemia and the removal of LDL by the liver represents one from the most important mechanisms regulating the level of plasma LDL. [28].

The increased triglycerides, total cholesterol were strongly supported. They found marked increase of serum triglycerides, cholesterol and LDL-cholesterol of abnormal lipid profile known as dyslipidemia may be the main cause of increase risk of cardiovascular disease as evidenced by atherosclerosis and increased body weight which is characterized by low HDL-c, raised triglycerides and a predominance of small, dense LDL-c particles and increase in free fatty acids FFA[19,30]. Diabetes mellitus is known as an important factor in hyperlipidemia determination in patients and may be due to hypertriglyceridemia which were observed in NIDDM and associated with hepatic over production of triglycerides and vLDL-c and impaired clearance of triglycerides rich lipoproteins. The hepatic over production of triglycerides is probably a consequence of increased flux of glucose and free fatty acids [30].

Effect on sex hormone:

Treatments of STZ-diabetic rats with various treatments afforded significant increase in serum testosterone level along the course of the study when compared with STZ diabetic rats. Treatments of diabetic rats with *N. sativa* afforded a marked increase in serum total testosterone which was greater than that of buffer group along the entire period of the experiment.

The L.H. was significantly decreased in STZ diabetic group along the 2nd and 4th weeks post STZ-treatment, whereas, a slight decrease was achieved after the first and 3rd week when compared with buffer group (Table 8) Treatment of diabetic rats with Rosiglitazone afforded a marked elevation in serum L.H along the entire experimental period when compared with buffer group.

Treatment of STZ diabetic rats with either silymarin or N.sativa afforded non-significant changes in serum L.H. along the course of the study when compared with their buffer group except diabetic group which showed a significant decrease in serum L.H. level after first week post drug administration compared with STZ non-treated group.

Whereas, the combination of Rosiglitazone with either *N.sativa* or silymarin elicited a significant elevation in serum L.H. level along the course of the study. When compared with STZ – diabetic group.

Our results were supported with, [16], they recorded that diabetes mellitus is commonly associated with reproductive neuro-endocrinopathy in both humans and animal models. Since the disease of diabetes is associated by reproductive failure in the males as a result of multi level days function within the hypothalamus, pituitary and testicular axis. Moreover, it has been reported that the level of L.H., F.S.H. and testosterone are significantly decreased in men with type II diabetes than in non-glycemic men [23, 24]. From the obtained results we can recommend the use of the combination of (Rosiglitazone + Silymarin) and

Zagazig J. Pharm. Sci., June, 2010
Vol. 19, No. 1, pp. 24-34

ISSN 1110-5089

(Rosiglitazone + *N. sativa*) which is known as a hepatoprotective drug in treatment of diabetic patients to avoid the proven hazardous effect of Rosiglitazone on liver, lipid profile as well as on male and female fertility. It was appearant that Rosiglitazone drug is not an ideal antidiabetic drug, since it showed many side effects represented by high level of Triglycerides, Total cholesterol, LDL-c,HDL-c ,VLDL-c as well as high level of F.S.H, L.H and decreased level of testosterone.

REFERENCES

1. Khanam, M. and Dewan, Z.F: **J. of Bangladesh Pharmacological Society**, 4: 17-20 (2008)
2. Kar, A.; Choudhary, B.K. and Bandyopadhyay, N.G: **J. of Ethnopharmacology**; 84:105-108 (2003).
3. Helmstadter, A.: **Pharmazie**, 62:717-720 (2007).
4. Abo, K.A.; Fredjalyesimi, A.A and Jalyesimi, A.E.: **J. of Ethnopharmacology**. 115:67-71 (2008)
5. Nehlin, J.: Department of Clinical Immunology, Odense University Hospital and University of Southern Denmark, 5000, Odens, Denmark. (2008).
6. Stephanie Saul: FDA Issues Safety Alert on Rosiglitazone. **The New York Times**, Friday, 12 December P. 07-88 (2008).
7. WHO Expert Committee on Diabetes Mellitus: Second report. **WHO Tech Rep Ser**; 646:1-80 (1980)
8. Al-Hader, A.; Aqel, M.; and Hasan, Z.: **International J. of Pharmacognosy** 31, 96-100 (1993)
9. Deresinski, S.: **Infect .Dis .Rep**; 1:1-12 (1995).
10. Desmet, P.A : **Drugs**; 54:801-840 (1997).
11. Friedman, M . and Mclellan, A.:.**ecnm press**; 272 pp (2006)
12. Kim, S.H.; Hyun, S.H. and Choung, S.Y.: **J. Ethnopharmacol** In press vol (39):20-25 (2006).
13. Marles, R.T. and Farnsworth, N.R.: **Phytomedicine**, 2: 137-189 (1995).
14. PDR for Herbal Medicines: Montvale,N J:Medical Economics Company. P: 516-520 (2000).
15. Gazioano, T.A.; Galea, G., and Reddy, K.S.: **Lancet**; 370:1939-1946 (2007).
16. Greenlee, H.; Abasca, K.; Yamerl, E. and Ladas, E.: **Intergrative. Cancer therapies** 6: 158-165 (2007).
17. Fossati, P. and Prencipe, L.: **Clin. Chem**; 6 (28):2077-2079 (1990).
18. Richmond, W.: **Amer . J. Clin .Chem** ; 19 :1350-1356 (1973) .
19. Stein, E.A: Textbook of Clinical Chemistry. NWTietz, ed., W.B Saunders, Philadelphia .PP .879-886. (1986).
20. Pierce, J.G., and Parson, T.F.: **Ann .Rev. Biochem**; 13: 450-465 (1981).
21. Carleton, H.M.: Carleton's Histological Technique. (4th Ed), Pub .London, New York, Toronto, **Oxford University Press** (1967).
22. Snedecor, G.W., and Cochran, W.G.: Statistical Methods (8th Ed), Ames Iowa State University (1982).
23. Szilard, S.; Szentgyorgyi, G. and Dhanalakshmi, S.: **Ada Med.** 45:249-256 (1988).
24. Labhal, A., Settaf, A., Bennani, N., Cherrah, Y., Slaoui, A., and Hassar ,M: **Espérance médicale** ;47:72-74 .(1997) .
25. Gruenwald, J.: Herbal Medicines (3rd Ed), Montvale, NJ: Thomson **PDR.Diabetologia**, 18:441- 446 (2004).
26. Yadiv, H.; Mukesh, A. and Shalm, Z.: **J. Pharmacol Sci** (10): 12-21 (2007).
27. Abbas, A.: Ph.D. Thesis (Pharmacology) presented to Faculty of Vet. Med., Zagazig University(2009).
28. Chopra, R.N.; Chopra, L.C.; Handa, K.L., and Kapoor, L.D.: Chopra's Indigenous drug of India, U.N. Dhar & Sons Pvt. Ltd. Calcuta, 2nd Ed (1958).
29. Satyanarayana, S.A.: **Ind. J. Pharm.**, 37.126 (1975).
30. El-Dakhakhny, M., Barakat, M., Abd-El-Halim, M., and Aly, S.M : **J. of Ethnopharmacology** 72, 299-304 (2000).

Received: March, 24, 2010
Accepted : June, 01, 2010

الدور التحسينى المحتمل لبعض المركبات على الآثار الجانبية لعقار الروزيجليتازون

منصور حسن زهرة ، أحمد عبد الحميد هنداوى وريهام زكريا حمره

قسم علم الحيوان – كلية العلوم – جامعة الزقازيق – الزقازيق – مصر

لقد تم دراسة الدور التحسينى المحتمل لبعض المركبات على الأثار الجانبية لعقار الروزيجليتازون تقسم الجرذان الى سبعة مجموعات فرعية كل واحدة من ١٠ فئران.

(١) المجموعة الأولى: أعطيت محلول متعادل وتركت كمجموعة ضابطة.

(٢) المجموعة الثانية: أعطيت استربتوزوتوسين عن طريق الحقن داخل الغشاء البريتوني وذلك لاحداث مرض السكر تجريبيا وتركت بدون علاج كمجموعة ضابطة.

(٣) المجموعة الثالثة: مصابة بمرض السكر تجريبيا وتم علاجها بالأفنديا بجرعة قدرها ٠,٥٨مجم/١٠٠ جرام من وزن الجسم يوميا ولمدة ٢٨ يوما متتالية عن طريق الفم.

(٤) المجموعة الرابعة: مصابة بمرض السكر تجريبيا وتم علاجها بالسليمارين يوميا ولمدة ٢٨ يوما متتالية عن طريق الفم بجرعة قدرها ٠,٥مجم/١٠٠ من وزن الجسم.

(٥) المجموعة الخامسة: مصابة بمرض السكر تجريبيا وتم علاجها بحبة البركة بجرعة قدرها ٠,٢٥جرام/١٠٠جرام من وزن الجسم يوميا ولمدة ٢٨ يوم.

(٦) المجموعة السادسة: مصابة بمرض السكر تجريبيا وتم علاجها بخليط الأفنديا مع حبة البركة بنفس الجرعات السابقة ولنفس المدة.

(٧) المجموعة السابعة: مصابة بمرض السكر وتم علاجها بخليط الأفنديا مع السليمارين بنفس الجرعات السابقة ولنفس المدة. ثم تجميع عينات دم من كل فأر بعد نهاية الأسبوع الأول، الثاني، الثالث والرابع من نهاية العلاج. وذلك لقياس نسبة الدهون المختلفة في البلازما وكذا قياس نسبة الهرمونات.

وأظهرت نتائج الدراسة الآتي:

التأثير على صورة الدهون:

أدى إحداث السكرى تجريبيا إلى حدوث زيادة معنوية في مستوى الجلسيريدات الثلاثية طوال الأسابيع الأربعة من نهاية العلاج عند مقارنتها بمجموعة الضوابط المعالجة بالمحاليل المتعادلة. بينما أدى علاج جميع المجموعات المصابة بمرض السكرى تجريبيا بالعلاجات المختلفة إلى حدوث نقص معنوي في مستوى الجلسيريدات الثلاثية طوال فترة التجربة عند مقارنتها بمجموعة الضوابط المصابة بمرض السكر فيما عدا المجموعة المعالجة بالأفنديا بعد الأسبوع الرابع من نهاية العلاج والتى أظهرت تأثيرات غير معنوية.

أظهرت المجموعة المصابة بالسكرى تجريبيا زيادة معنوية في مستوى الكولستيرول الكلى طوال فترة التجربة عند مقارنتها بمجموعة الضوابط المعالجة بالمحاليل المتعادلة بينما أدى علاج المجاميع المختلفة بالعلاجات المختلفة إلى حدوث نقص معنوي في مستوى الكوليسترول الكلى طوال فترة التجربة عند مقارنتها بمجموعة الضوابط المصابة بمرض السكر.

أحدث السكرى المستحدث تجريبيا إلى حدوث تغيرات غير معنوية في مستوى الكولسترول عالى الكثافة طوال فترة التجربة ولقد أحدثت العلاجات المختلفة للمجاميع المصابة بالسكرى تجريبيا تأثيرا غير معنويا فى مستوى الكولستيرول عالى الكثافة طوال فترة التجربة فيما عدا المجموعة المعالجة بالأفنديا بعد نهاية الأسبوع الأول من نهاية العلاج والتى أظهرت زيادة معنوية في مستوى الكولسترول عالى الكثافة عند مقارنته بمجموعة الجرذان المصابة بالسكرى والغير معالجة. وكذا المجموعة المعالجة بالسليمارين بمفرده بعد الأسبوع الثالث والرابع والمجموعة المعالجة بخليط الأفنديا + حبة البركة بعد الأسبوع الثالث والمجموعة المعالجة بالأفنديا والسليمارين بعد الأسبوع والرابع والتى أظهرت نقصا معنويا.

أما بالنسبة للكولسترول منخفض الكثافة فقد أحدث السكرى المستحدث تجريبيا ارتفاعا معنويا في مستوى الكولسترول منخفض الكثافة في طوال فترة التجربة عند مقارنته بمجموعة الضوابط المعالجة بالمحاليل المتعادلة بينما أدى علاج جميع المجموعات المصابة بالسكرى بالعلاجات المختلفة إلى حدوث نقص معنوي في مستوى الكولسترول منخفض الكثافة طوال فترة التجربة عند مقارنته بمجموعة الضوابط المصابة بالسكرى والغير معالجة.

بالنسبة للكولسترول شديد الإنخفاض في الكثافة أدت إصابة الجرذان بالسكرى المستحدث تجريبيا إلى حدوث ارتفاع معنوي في مستوى الكولستيرول شديد الانخفاض في الكثافة طوال فترة التجربة عند مقارنتة بمجموعة الضوابط المعالجة بالمحاليل المتعادلة. ولقد أدى علاج جميع المجموعات المصابة بالسكرى بالعلاجات المختلفة إلى حدوث انخفاض في مستوى الكولستيرول انخفاض في الكثافة طوال فترة التجربة عند مقارنتة بمجموعة الضوابط المصابة بالسكرى.

التأثير على الهرمونات الجنسية:

التأثير على هرمون التستوستيرون :

أدى إحداث السكرى تجريبيا إلى حدوث نقص معنوي في مستوى التستوستيرون طوال فترة التجربة عند مقارنته بمجموعة الضوابط المعالجة بالمحاليل المتعادلة. بينما أدى علاج جميع المجاميع المصابة بالسكرى بالعلاجات المختلفة الى حدوث تغيرات غير معنوية في مستوى هرمون التستوستيرون طوال فترة التجربة فيما عدا المجموعة المعالجة بالأفنديا وحبة البركة كل على حدة والتى أظهرت نقصا وزيادة معنوية في مستوى الهرمون على التوالى طوال فترة التجربة عند مقارنته بمجموعة الضوابط المصابة بالسكرى.

بالنسبة للهرمون FSH :

أما بالنسبة للهرمون المؤثر على نمو البويضات FSH فقد أدى إحداث السكرى المستحدث تجريبيا إلى حدوث نقص معنوي في مستوى الهرمون الحادث على نمو البويضات طوال الأسابيع الثلاثة الأخيرة عند مقارنتة بمجموعة الضوابط المعالجة بالمحاليل المتعادلة.

بينما أدى علاج جميع المجاميع المصابة بالسكرى المحدث تجريبيا بالعلاجات المختلفة إلى حدوث إرتفاع معنوي في هذا الهرمون طوال فترة التجربة عند مقارنته بمجموعة الضوابط المصابة بالسكرى كما حدث نقص معنوي في مستوى هذا الهرمون

ISSN 1110-5089

Zagazig J. Pharm. Sci., June, 2010
Vol. 19, No. 1, pp. 24-34

طوال فترة التجربة في المجموعة المعالجة بالسليمارين أو حبة البركة كل على حدة فيما عدا بعد الاسبوع الاول في المجموعة المعالجة بحبة البركة والتى أظهرت نقصا غير معنويا. بينما أدى إعطاء خليط الأفنديا مع كل من حبة البركة أو السليمارين إلى حدوث تغير غير معنوي طوال فترة التجربة عند المقارنة بمجموعة الضوابط المصابة بالسكرى.

أما بالنسبة للهرمون L.H:

أدى إحداث السكرى المستحدث تجريبيا إلى نقص معنوي في مستوى الهرمون الحاث على التبويض طوال فترة التجربة عند مقارنته بمجموعة الضوابط المعالجة بالمحاليل المتعادلة بينما أدى علاج المجموعات المصابة بالسكرى بالافنديا بمفرده أو خليط منه مع السليمارين إلى حدوث زيادة معنوية في مستوى هذا الهرمون في الدم طوال فترة التجربة عند مقارنة ذلك بمجموعة الضوابط المصابة بالسكرى. ولكن علاج جميع المجاميع المصابة بالسكرى بالسليمارين أو حبة البركة كل على حدة أو خليط من الأفنديا مع حبة البركة أحدث تأثيرات غير معنوية في مستوى هذا الهرمون في الدم طوال فترة التجربة عند المقارنة بمجموعة الضوابط المصابة بالسكرى.