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Possible ameliorative role of some compounds on the side effects of *Avandia* (Drug)

ABD EL-AZIZ A. DIAB, MANSOUR H. ZAHRA, AHMED A. HENDAWY, SAMIH, I. EL-DAHMY* and REHAM Z. HAMZA

Department of Zoology, Faculty of Science, Zagazig University, Cairo (Egypt).
*Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy, Zagazig University, Cairo (Egypt).

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ABSTRACT

The present study was carried out to evaluate the hypoglycemic effect of Avandia, Nigella sativa, Silymarin each alone and the combination of Avandia with either Nigella sativa or Silymarin In order to get the best combination to avoid the possible side effects produced by Avandia. This was done through studying the effect of the test plant drugs and their combination on serum glucose, insulin , some liver and kidney function parameters .seven groups of adult male rats each of 10 (200-250 gm / b.wt) were used in this study . Hyperglycemia was induced in six groups of rats. Whereas, the seventh group was left as normal control group. All treatments were given orally daily for successive 28 days .The first group was left without treatment and kept as STZ diabetic. The 2nd group was administered Avandia (0.58mg/100gm.b.wt) , The third group was given Nigella sativa(0.25gm/ 100gm.b.wt) , the 4th group was given Silymarin (50mg/100gm.b.wt), The 6thand 7th groups were administered the combination of Avandia 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 Avandia afforded a marked increase in serum ALT, AST, LDH activities, Serum urea, Uric acid and creatinine as well a significant elevation in blood glucose level and significant decrease in serum insulin level. Treatment of diabetic rats with various treatments elicited a marked decrease in serum ALT,AST,LDH, Urea, Uric acid and creatinine as well as a marked decrease and increase in blood glucose and serum insulin respectively when compared with diabetic non treated group .The best results was obtained with the combination of Avandia+ Silymarin on blood sugar and insulin levels.

Key word: *Nigella sativa,* Avandia and Anti diabetic drug.

INTRODUCTION

The increasing prevalence of diabetes mellitus in the present day world is a cause of concern to the mankind. Diabetes mellitus, whether of type I or type II category, is primarily characterized by either lack of insulin or its action which starts with derangement of carbohydrate metabolism to eventually entangle derangement of protein and lipid metabolism[1].

Diabetes is a chronic disease characterized by disordered metabolism and in appropriately high blood sugar (hyperglycemia). Type 2 diabetes is sharply increasing globally, including many parts of the developing world, in major part as a consequence of the world wide "epidemic" of obesity, prior to and after the discovery of insulin, medicinal plants have been used to normalize glycemia in diabetic patients[2,3,4].

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 secregatogues, either in

single therapy or in combination, would help to improve glycemic control, either by increasing peripheral glucose uptake, improving insulin secretion, decreasing hepatic glucose output or reducing the influx of glucose to the body[5].

Avandia manufactured by Glaxo Smithkline (GSK), was approved as an adjunct to diet and exercise to improve control of blood sugar levels. Avandia is approved to be used as a single therapy or used in combination with metformin and sulfonylurea, or with other oral anti-diabetes treatments[6].

The WHO expert committee on diabetes mellitus recommendations of 1980[7] included investigation of hypoglycemic agents from plants used in traditional medicine. *Nigella sativa* oil have been used for treatment of experimentally induced dia-betes in animals based on its combined hypoglycemic and immunopotentiating effects that help in ameliorating the impaired immunity and infections associated with diabetes[8,9].

Increased utilization of medicinal plants became a World Health Organization (WHO) policy in 1970. Plants and herbs are chemical factories that directly provide about 25% of currently used drugs and another 25% of drugs comprise chemically altered natural products[10].

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

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 in ability of current therapies for many rural populations, particularly in developing countries[13].

One of the important issues regarding silymarin is that it may be accepted as a safe herbal product since no health hazards or side effects are known in connection with the proper administration of designed therapeutic dosages[14]. We therefore planned to investigate the insulinotropic effects of extract of natural product of *N. sativa* seeds and Silymarin on blood glucose and insulin levels of serum in adult male albino rats and its role in reducing the side effects of Avandia drug together with studying their effects on some liver and kidney function parameters.

EXPERIMENTAL

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

Induction of diabetes:

(STZ diabetic groups). 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 group

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

Animals were given a daily oral dose of AVA (0.58 mg/100g.b.wt) dissolved in 1 ml Tragacanth gum as suspension for 4 weeks.

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

Animals were received a daily oral dose of *Nigella sativa* extract (0.25gm/100g b.wt) for 4 weeks.

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

Animals were given dose of Silymarin extract (50mg/kg.b.wt) suspended in 1 ml CMC suspension orally for 4 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 for4 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 4 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 for4weeks.

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 heparinized microhaematocrit capillary tubes into centrifuge tubes. Serum was harvested from blood without anticoagulant and used for determination of serum ALT & AST[15], Lactic dehydrogenase[16], Urea[17], Creatinine[18], Uric acid[19], glucose[20] and serum insulin was assayed using insulin – I^{125}.
Kit [21] using radioimmunoassay kit supplied by Radioassay system laboratory Inc (England).

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 AND DISCUSSION

The results of experiment revealed the following observations.

(1) Effect on some liver function parameters:

(A) Effect on serum Alanine amino transferase (ALT) activity

Table (1) revealed that the induction of diabetes in rats by STZ elicited a marked increase in serum ALT along the entire period of the study when compared with control group.

The administration of various drugs & their combinations for 28 days to diabetic rats afforded a significant decrease (P< 0.05) in serum ALT activity along the entire period of the experiment when compared with STZ treated group while administration of Avandia elicited highly significant increase in serum ALT activity when compared with control group.

(B) Effect on serum Aspartate amino transferase (AST) activity

Our results revealed that STZ afforded a marked increase in serum AST activity (P< 0.05) along the entire period of the study when compared with control group.

All treatments of diabetic rats elicited a marked decrease (P< 0.05) in serum activities of AST when compared with STZ except treating with Avandia showed marked increase in serum AST activity.

Diabetic rats along the course of the study except groups treated with Silymarin, *Nigella sativa* and combination of Avandia+ *Nigella sativa* after 3rd week and group treated with Avandia+ Silymarin after 4th week post drug administration which showed non significant changes (Table 2)

(C) Effect on serum lactic dehydrogenase enzyme (LDH) activity

STZ diabetic group showed significant increase in serum LDH activity along the entire period of the experiment when compared with control group. Whereas all treatments of diabetic rats for 28 days afforded a marked decrease (P< 0.05) in serum LDH activity along the course of the study when compared with STZ treated group except group treated with Avandia showed highly significant increase in LDH activity when compared with control group (Table 3) .

(2) Effect on Urea, Uric acid and Creatinine:

Induction of diabetics by STZ induced a significant increase in serum urea, uric acid and creatinine along the entire period of the study when compared with control group.

Treatments of diabetic rats with various treatments elicited a significant decrease in serum urea, uric acid and creatinine along the course of the experiment when compared with STZ diabetic group except group treated with Avandia showed

highly significant increase in serum urea, uric acid and creatinine when compared with control group (Table 4, 5, 6).

(3) Effect on blood glucose levels:

(A) Effect on serum Glucose

Concerning the effect of test drugs and their combinations on serum glucose level of diabetic rats, (table 7) showed that STZ afforded a marked elevation in serum glucose when compared with control group along the course of the study, whereas all treated groups revealed a significant decrease ($P<0.05$) in serum glucose level when compared with STZ non-treated group along the entire course of the experiment with the rank order of potency as antidiabetic as follows: Avandia + Silymarin > Silymarin > Avandia + *Nigella sativa* > Avandia > *Nigella sativa*. After the end of the experiment (4 weeks).

(B) Effect on serum Insulin

(Table 8) reveals that STZ afforded a marked decrease in serum insulin of rats ($P< 0.05$) when compared with control group along the 4 weeks of the experiment. Whereas, administration of Avandia for 4 weeks elicited non-significant change in serum insulin level of diabetic rats when compared with STZ diabetic group along the course of the study. The other treatments of the diabetic rats for 4 weeks elicited a significant increase ($P< 0.05$) in serum level of insulin along the entire period of the experiment except with silymarin after the first week which revealed non-significant increase when compared with STZ treated group.

DISCUSSION

The present study was an attempt to evaluate the hypoglycaemic effect of Avandia, *Nigella sativa*, silymarin each alone and the combination of Avandia with either N.sativa or silymarin when given to normal and diabetic rats for 28 successive days. Their effects on some on some liver function parameters (ALT, AST, and LDH) were also studied. Some kidney function parameters (serum urea, uric acid and creatinine), lipogram as well as the effect on insulin were also investigated).

Because of low cost, traditional medicinal plants also raise significant interest to prevent morbidity and mortality from chronic diseases where low or middle income populations are important[23].

Effect on liver function parameters:

Our results showed that Avandia, Silymarin, *Nigella sativa* and their combination on when given daily for successive 28 days afforded a significant decrease in serum ALT activity along the entire period of the experiment in hyperglycemic rats when compared with STZ group.

Our result seems to be conceivable with that previously reported[24, 25]. They reported that milk thistle have a protective effects on the liver and greatly improve its function since it is typically used to treat liver cirrhosis and chronic hepatitis (liver inflammation).

In addition, milk thistle extracts both prevent and repair damage from toxic chemicals and medications. Workers who had been exposed to vapors from toxic chemicals for 5 – 20 years were given silymarin for 30 days. They showed significant improvement in liver function tests (ALT & AST) and platelet counts[26]. The liver regenerating effect induced by silymarin results from stimulation of RNA polymerase enzyme in the nucleus of liver cells. This results in an increase of ribosomal protein synthesis which helps to regenerate hepatocytes[27]. On a similar ground, silymarin might also affect bone marrow.

Our results were in full agreement with Yadiv *et al.*[28]. They reported that oral administration of rosiglitazone in diabetic patients significantly increased serum ALT and AST activities 2.5 times more than normal and leads to severe dysfunction, weight gain, oedema and milk dilutional anemia. Induction of diabetes by STZ afforded a significant elevation in serum ALT activity along the entire course of the study. A result which is supported by the results reported before[29]. She reported that the induction of diabetes by alloxan caused a significant elevation in serum ALT and AST along the course of the study (4 weeks). Treatments other than Avandia caused a significant reduction in the elevated serum transaminases activity along the entire course of the study when compared with STZ group alone. Indicating that these plant drugs succeeded in improving the status of injured liver

Table 1: Effect of Avandia (0.58 mg/100g.b.wt), Nigella sativa (0.25gm/100g b.wt), Silymarin (50mg/kg.b.wt) and their combinations on serum alanine amino transferase (ALT) concentration (U/ml) in diabetic male albino rats (mean±SE). (N =7)

	Groups	ALT (1st Week)	ALT (2nd Week)	ALT (3rd Week)	ALT (4th Week)
Diabetic	1. STZ (diabetic non treated group)	13.25+0.83d	12.50+0.66b	13.28+0.75b	12.33+0.93b
	2. STZ + Avandia Group	60.63+6.05a	64.62+5.96a	74.71+5.83a	78.48+4.60a
	3. STZ + Silymarin Group	11.76+0.74e	9.72+0.58e	9.26+1.40d	8.96+0.64d
	4. STZ + Nigella sativa Group	8.33+0.76f	8.66+0.65e	8.68+1.06e	8.66+0.35d
	5. STZ + Avandia + Nigella sativa Group	29.66+1.89b	25.62+1.27b	15.83+0.60b	14.30+0.76b
	6. STZ + Avandia + Silymarin Group	18.58+0.95c	15.95+1.18b	13.16+0.54b	12.00+0.73b
	7.Control group	12.16+0.70d	13.20+0.39b	12.43+0.49b	12.08+0.23 b

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

Table 2: Effect of Avandia (0.58 mg/100g.b.wt) , *Nigella sativa* (0.25gm/100g b.wt), Silymarin (50mg/kg.b.wt) and their combinations on serum aspartate amino transferase (AST) concentration (U/ml) in diabetic male albino rats (mean ± SE). (N =7)

	Groups	AST (1st Week)	AST (2nd Week)	AST(3rd Week)	AST(4th Week)
Diabetic	1. STZ (diabetic non treated group)	50.00+2.59b	52.16+3.20b	64.66+0.95b	60.83+1.07b
	2. STZ + Avandia Group	119.83+17.93a	122.79+18.88a	167.50+6.06a	175.50+6.18a
	3. STZ + Silymarin Group	58.63+2.51b	56.71+2.50b	25.66+4.26de	19.83+1.30e
	4. STZ + *Nigella sativa* Group	47.50+3.93c	44.66+4.21c	36.16+1.49d	44.66+4.21e
	5. STZ + Avandia + *Nigella sativa* Group	51.16+1.04b	44.50+1.40c	27.16+5.05de	15.50+4.24e
	6. STZ + Avandia + Silymarin Group	64.52+2.05b	54.37+2.63b	47.45+1.82e	28.16+1.42d
	7.Control group	34.93+0.68d	32.10+0.63d	34.16+1.01d	32.83+1.13d

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

Table 3: Effect of Avandia (0.58 mg/100g.b.wt), *Nigella sativa* (0.25gm/100g b.wt), Silymarin (50mg/kg.b.wt) and their combinations on serum lactate dehydrogenase enzyme activity (LDH) (µIU/ml) in diabetic male albino rats (mean ± SE). (N =7)

	Groups	LDH (1st Week)	LDH (2nd Week)	LDH (3rd Week)	LDH (4th Week)
Diabetic	1. STZ (diabetic non treated group)	80.5.00+106.45c	769.33+101.00c	736.66+9780bc	704.66+80.80bc
	2. STZ + Avandia Group	2526.66+356.48a	2855+00+286.84a	3266.66+414.86a	3394+33+300.98a
	3. STZ + Silymarin Group	406.66+22.01d	373.33+26.19d	350.00+27.23d	1141.13+121.74b
	4. STZ + *Nigella sativa* Group	372.00+30.68d	341.00+19.85d	314.16+23.46d	286.83+18.83d
	5. STZ + Avandia + *Nigella sativa* Group	847.33+76.85c	804.1681.95c	746.33+91.74bc	706.83+54.87bc
	6. STZ + Avandia + Silymarin Group	1437.66+200.15b	1381.66+279.19b	1296.66+274.7b	1141.33+121.74b
	7.Control group	290.50+14.45d	352.16+14.10d	341.00+13.29d	334.16+13.02d

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

Table 4: Effect of Avandia (0.58 mg/100g.b.wt) , *Nigella sativa* (0.25gm/100g b.wt) and Silymarin (50mg/kg.b.wt) and their combinations on serum urea concentration (mg/dl) in diabetic male albino rats (mean ± SE). (N =7)

	Groups	Urea (1st Week)	Urea (2nd Week)	Urea (3rd Week)	Urea (4th Week)
Diabetic	1. STZ (diabetic non treated group)	24.96±0.77e	25.45±0.91e	37.50±0.71b	35.00±0.63b
	2. STZ +Avandia Group	79.77±4.79a	84.17±5.34a	100.88±2.43a	124.45±11.83a
	3. STZ +Silymarin Group	56.52±3.84b	36.19±2.58bc	34.70±2.59bc	30.11±2.69b
	4. STZ + *Nigella sativa* Group	45.50±2.13c	40.53±1.84b	32.20±3.77bc	29.03±4.97b
	5. STZ + Avandia + *Nigella sativa* Group	32.44±1.91d	31.74±1.82bc	27.83±1.90c	27.00±2.12b
	6. STZ +Avandia+ Silymarin Group	39.08±1.88d	28.33±2.23cd	27.97±2.25c	27.99±1.85b
	7.Control group	33.33±3.24d	38.98±3.39b	33.05±0.97bc	37.28±1.84b

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

Table 5: Effect of Avandia (0.58 mg/100g.b.wt) , *Nigella sativa* (0.25gm/100g b.wt), Silymarin (50mg/kg.b.wt) and their combinations on serum uric acid concentration (mg/dl) in diabetic male albino rats (mean ± SE). (N=7)

	Groups	Uric acid (1stWeek)	Uric acid (2nd Week)	Uric acid (3rd Week)	Uric acid (4th Week)
Diabetic	1. STZ (diabetic non treated group)	3.56±0.30b	3.51±0.27b	3.18±0.64b	2.55±0.55bc
	2. STZ +Avandia Group	7.93±0.99a	10.86±2.40a	11.84±1.55a	11.59±1.17a
	3. STZ + Silymarin Group	2.53±0.17c	2.27±0.13bc	1.65±0.21c	1.48±0.19c
	4. STZ +*Nigella sativa* Group	4.31±0.22b	3.50±0.11b	2.99±0.23b	3.50±0.11b
	5. STZ+ Avandia + *Nigella sativa* Group	3.55±0.37b	2.89±0.28b	2.01±0.23b	1.77±0.25c
	6. STZ + Avandia +Silymarin Group	3.29±0.18b	2.77±0.28b	2.11±0.18b	1.63±0.19c
	7.Control group	1.95±0.06d	1.88±0.13c	1.72±0.23c	1.96±0.25c

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

Table 6: Effect of Avandia (0.58 mg/100g.b.wt) , *Nigella sativa* (0.25gm/100g b.wt), Silymarin (50mg/kg.b.wt) and their combinations on serum creatinine concentration (mg/dl) in diabetic male albino rats (mean ± SE). (N=7)

	Groups	creatinine (1stWeek)	creatinine (2nd Week)	creatinine (3rd Week)	creatinine (4th Week)
Diabetic	1. STZ (diabetic non treated group)	0.97±0.05e	0.84±0.03d	1.37±0.04c	1.15±0.02c
	2. STZ + Avandia Group	2.92±0.18a	3.45±0.18a	3.71±0.17a	4.12±0.39a
	3. STZ + Silymarin Group	1.67±0.12c	1.49±0.15c	1.14±0.08d	1.17±0.10c
	4. STZ + *Nigella sativa* Group	1.46±0.04cd	1.33±0.06c	1.37±.06c	0.95±0.11cd
	5. STZ + Avandia + *Nigella sativa* Group	1.25±0.06de	0.97±0.08d	1.25±0.06d	0.89±.007cd
	6. STZ + Avandia + Silymarin Group	1.21±0.05de	0.90±0.05d	1.33±0.03c	0.89±.0.06d
	7.Control group	2.09±0.10b	1.95±0.14b	2.02±0.03b	1.90±.06b

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

Table 7: Effect of Avandia (0.58 mg/100g.b.wt) , *Nigella sativa* (0.25gm/100g b.wt), Silymarin (50mg/kg.b.wt) and their combination on serum glucose concentration (mg/dl) in diabetic male albino rats. (mean ± SE). (N=7)

	Groups	Glucose (1st Week)	Glucose (2nd Week)	Glucose (3rd Week)	Glucose(4th Week)
Diabetic	1. STZ (diabetic non treated group)	398.33+11.08a	441.00+15.13a	454.16+17.72a	466.10+30.05a
	2. STZ + Avandia Group	190.33+16.03c	185.66+17.88c	148.33+11.45c	185.66+17.88c
	3. STZ + Silymarin Group	150.83+19.71cd	130.55+17.89d	109.36+12.56de	84.33+4.55d
	4. STZ+ *Nigella sativa* Group	272.50+10.15b	280.16+7.44b	330.00+17.98b	321.66+17.96b
	5. STZ + Avandia + *Nigella sativa* Group	178.33+22.90cd	166.33+20.84cd	125.83+6.88cd	110.11+8.19d
	6. STZ +Avandia + Silymarin Group	144.66+6.30d	138.16+6.36d	119.16+3.96cde	92.83+9.56d
	7.Control group	111.83+0.87e	106.67+2.94e	105.30+2.30e	108.70+1.95d

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

Table 8: Effect of Avandia (0.58 mg/100g.b.wt), *Nigella sativa* (0.25gm/100g b.wt), Silymarin (50mg/kg.b.wt) and their combinations on serum insulin levels (IU/ml) in diabetic male albino rats. (mean ± SE). (N=7).

	Groups	Insulin (1st Week)	Insulin (2nd Week)	Glucose (3rd Week)	Glucose(4th Week)
Diabetic	1. STZ (diabetic non treated group)	14.23+1.15d	14.79+0.89e	15.80+0.86e	16.63+0.96f
	2. STZ + Avandia Group	18.35+2.67cd	16.28+1.93de	14.57+1.29e	11.43+0.69f
	3. STZ + Silymarin Group	18.70+2.47cd	22.08+2.85cd	23.88+2.91d	32.17+2.22d
	4. STZ +*Nigella sativa* Group	38.33+1.94ab	41.16+1.90b	41.00+1.98ab	40.83+1.43b
	5. STZ +Avandia +*Nigella sativa* Group	24.66+2.02c	28.16+2.10c	30.33+2.38c	38.27+2.68bc
	6. STZ + Avandia +Silymarin Group	19.0+0.49c	20.01+1.48d	22.33+0.95d	23.99+1.01e
	7.Control group	42.33+3.22a	47.66+2.28a	45.00+2.08a	47.16+2.68a

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

caused by STZ. It has been reported that thymoquinone, one the active constituents of N.sativa have a hepatoprotective activity. An in vitro study showed the protective effect against tert-butyl hydroperoxide (TBHP) induced oxidative damage to hepatocytes. The activity was demonstrated by a decreased leakage of ALT, AST and decreased trypan blue uptake[30,31].

The hepato protective effect of N.sativa was supported also by the results of El-Dakhakhny *et al.,*[32], and Mahmoud *et al.,* [33]. The same previous effects were observed on serum AST level with the exception of effects of *N. sativa*, Silymarin and their combination with Avandia which showed non-significant changes when compared with STZ group. This may be attributed to the fact that AST is not a liver specific enzyme.

Our results coincide also with Szilard, et al. [26] they reported that milk thistle and both extracts repair damage caused by toxic chemicals and medications. They exposed workers to vapors from toxic chemicals (Toluene and / or xylene) for 5-20 years, and were given either a standardized milk thistle (80% silymarin) for 30 days. The workers showed significant improvement in liver function tests (ALT & AST) when compared with placebo.

On the same basis, milk thistle is used primarily to treat various liver diseases and dysfunctions including alcoholic cirrhosis, hepatitis (due to viral infection or drug – induced) as well as hepatic problems related to diabetes[34-38].

Silymarin has liver regenerative effects by stimulating the enzyme known as RNA Polymerase in the nucleus of liver cells. This results in an increase of ribosomal protein synthesis which helps to regenerate hepatocytes[27].

All treatments of diabetic rats for 28 days afforded a marked decrease in serum LDH activity alo ng the entire course of the study when compared with STZ diabetic group.

These effects could be discussed in the same manner as with ALT and AST as previously mentioned.

Effect on Kidney functions parameters:

Concerning the effects of the test plant drugs on the kidney function parameters,Our results revealed that serum urea, uric acid and creatinine were significantly elevated in serum of STZ – diabetic rats compared to control group. Our results coincides with Jones *et al.,* [39]. They recorded that creatinine is increased in diabetic rats , they attributed this increase due to the deterioration of renal function induced by diabetes. Their findings were supported by the degenerative changes observed in the kidney in this study. While it has been found that serum creatinine is increased significantly in IDDM patients[40].

The functional abnormalities in diabetic kidneys due to the increase in glomerular filtration rate (GFR) were attributed to the increase in both glomerular capillary pressure and flow [41]. Treatment of diabetic rats with various treatments afforded a significant decrease in serum urea, uric acid and creatinine when compared with STZ diabetic group along the course of the study . Our results were reinforced with the results of Ledi *et al.,* [42]. They found that treatment of diabetic rats with rosiglitazone (Avandia) showed a significant decrease in urea, uric and creatinine by decreasing creatinine kinase. The other drugs may produce their effect also through this mechanism.

Effect on Glucose level:

Concerning the effect of the test plant drugs on serum glucose level in diabetic rats. It is a well known fact that 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 or insulin action[43].

The STZ treated group showed a marked increase in serum glucose level along the entire period of the experiment when compared with control group. These results were supported by the decreased insulin level in the STZ treated group and the significant increase in insulin level in all treated groups along the course of the study when compared with diabetic non-treated groups.

It has been reported that insulin is a hormone that has extensive effects on metabolism

and other body functions, such as vascular compliance. Insulin causes cells in the liver, muscle and fat tissue to take up glucose from the blood storing it as glycogen in the liver and muscle and supporting use of fat as an energy source. When insulin is absent (or low), glucose is not taken up by body cells, and the body begins to use fat as an energy source for example by transfer of lipids from adipose tissue to the liver for mobilization as an energy source. As its level is considered control metabolic control mechanism, its status is also used as a control signal to other body systems (such as amino acid uptake by body cells). It has several other anabolic effects throughout the body. When control of insulin levels fails, diabetes mellitus results(44).

Our results were also in accordance with Guyton and Hall [45]. They reported that most of the pathology of diabetes can be attributed to one of the following 3 major effects of insulin lack.

(a) Decreased utilization of glucose by the body cells, with a resultant increase in blood glucose concentration 300–1200 mg/dl.
(b) Markedly increased mobilization of fats from the fat storage areas, causing abnormal fat metabolism as well as deposition of lipids in vascular walls to cause atherosclerosis. A fact which is supported by the increased serum level of triglycerides and total cholesterol and its fraction LDL-C and VLDL-C.
(c) Depletion of protein in the tissues of the body.

Moreover, it has been found that rosiglitazone treatment decreased blood glucose concentration, increased plasma insulin concentration and preserved pancreatic islet mass[46].

Thiazolidinediones increase glucose transport into muscle and adipose tissue by enhancing the synthesis and translocation of specific forms of the glucose transporter proteins[47], They added also that thiazolidinediones exert their principal effects by lowering insulin resistance in peripheral tissue, but an effect to lower glucose production by the liver has been also reported.

It has been recorded also that Rosiglitazone a thiazolidinedione with a different side chain from those of troglitazone and pioglitazone, reduces plasma glucose levels and glucose production and increases glucose clearance in patients with type 2 diabetes mellitus. Insulin sensitivity, pancreatic beta cell function and surrogate markers of cardiovascular risk factors are significantly improved by rosiglitazone (48). Our results were reinforced also by Wagstaff and Goa[49]. They recorded that rosiglitazone 4mg/day provides significant antihyperglycemic efficacy, and generally tolerate, both as monotherapy and in combination with other antihyperglycemic agents, in patients with type 2 diabetes mellitus who do not have active liver disease.

Furthermore, it has been shown that sulphonylureas and rosiglitazone significantly improved long term glycemic control by restoring insulin secretion and reduced postprandial hyperglycemia in peripatetic subjects and similarly effective in elderly by and non elderly populations with type 2 diabetes. In the same direction[50]. Rosiglitazone enhanced the insulin sensitivity in the liver, skeletal muscle and adipose tissue in type 2 diabetes[47,51].

TZD enhance insulin action and improve glycemic control by increasing peripheral glucose disposition and reducing hepatic glucose output through activation of PPAR-Y [52].

The hypoglycaemic effect of *N. sativa* observed in this study in diabetic rats together with the increased level of insulin was in full agreement with Al-Awadi *et al.,* [53], Al-Hader *et al.*[8]. Their results indicated that the volatile oil of N.sativa afforded a hypoglycaemic effect in diabetic rats and rabbits.

It has been also reported that treatment of STZ diabetic rats with the plant extracts (*N.sativa*) and (Silymarin) produce a significant increase of serum insulin level[54]. Recently, Benhaddou–Andaloussi *et al.,* [55] used in vitro bioassays to identify target tissues and demonstrate the insulinotroipic and insulin – like activities of an ethanol extract of Nigella sativa. Haddad *et al.,* [56], reported that *N. sativa* has been found to rank high among the antidiabetic plants, and most recommended by traditional practitioners. It has been also reported that *N. sativa* possesses a significant hypoglycemic activity which is though to be due to the essential

oil present (30&31). Eskander *et al.,*[57] recorded the hypoglycemic effect of herbal formulation of *N. sativa* plant in alloxan induced diabetic rats.

The hypoglycemic effect of the volatile oils of *N. sativa* was confirmed before[58]. They found that oral administration of the volatile oil of *N. sativa* to STZ – diabetic rats afforded a significant elevation in serum insulin level.

The hypoglycemic effect of Milk thistle (Silymarin) observed in this study in STZ diabetic rats is supported with the findings of (59). They stated that the extract of milk thistle (Silymarin) can help people to lower the amount of sugar bound to haemoglobin in blood, as well as reducing fasting blood sugar level. Silymarin is also effective in improving glycaemic profile in patients with type II diabetes.

CONCLUSIONS

From the obtained results, we report that Avandia drug is not an ideal antidiabetic drug, since it showed many side effects represented by high level of AST, ALT, Urea, Uric acid,Creatinine. Moreover, the combination of Avandia and Silymarin gave the best results on blood sugar level.

Recommendations

So we recommended the use of the combination of Avandia and Silymarin which is known as a hepatoprotective drug in treatment of diabetic patients to avoid the proven hazardous effect of Avandia on cardiovascular system and to overcome the side effects of Avandia on liver as well as on male and female fertility.

REFERENCES

1. Khanam, M. and Dewan, Z.F. : Effects of the crude and the n-hexane extract of Nigella sativa linn. (kalajira) upon diabetic rats. *A journal of Bangeldesh pharmacological socieity,* **4:** 17-20 (2008).
2. Kar, A.; Choudhary, B.K. and Bandyopadhyay, N.G. : Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *Journal of Ethnopharma-cology;* **84:** 105-108 (2003).
3. Helmstadter, A. : Antidiabetic drugs used in Europe prior to the discovery of insulin. *Pharmazie ;* **62:** 717-720 (2007).
4. Abo, K.A.; Fredjaiyesimi, A.A and Jaiyesimi, A.E. : Ethnobotanical studies of medical plants used in the management of diabetes mellitus in South western Nigeria. *Journal of Ethnopharmacology;* **115:** 67-71 (2008).
5. Nehlin, J. : Recent developments in the treatment of diabetes type 2, Department of Clinical Immunology ,Odense University Hospital and University of Southern Denmark. 5000, Odens, Denmark (2008).
6. Stephanie Saul : FDA Issues Safety Alert on Avandia., 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. : Hypoglycemic effects of the volatile oil of Nigella sativa seeds. *International Journal of Pharmacognosy* **31,** 96-100 (1993).
9. Deresinski, S. : Infections in the diabetic patient :Strategies for clinicans. *Infect .Dis .Rep;* **1:** 1-12 (1995).
10. Desmet, P.A. : The role of plant –derived drugs and herbal medicines in health care. *Drugs*; **54:** 801-840 (1997).
11. Friedman, M., and Mclellan, A. : Healing diabetes: Complementary naturopathic and drug treatments .ccnm press; 272 pp (2006).
12. Kim, S.H.; Hyun, S.H. and Choung, S.Y. : Antidiabetic effect of Cinnamon extract on blood glucose in db/db mice. *J. Ethnoph-armacol.* **39:** 20-25 (2006).
13. Marles, R.T. and Farnsworth, N.R. : Anti-diabetic plants and their active constituents. *Phytomedicine,* **2:** 137-189 (1995).
14. PDR for Herbal Medicines : Montvale ,NJ:Medical Economics Company. P:516-

520 (2000).

15. Reitman, S. and Frankel, S.: A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic transaminase. *Amer. J. Clin. Pathology.* **28:** 56 (1957).
16. Esakova, T.; and Ivanov, M. : Interaction of lactate dehydrogenase and Membranes of the sarcoplasmic reticulum. Russian, Biokhimiia. **57:** 253 (1992).
17. Patton, C. and Crouch, G. : Enzymatic colorimetric determination of urea. *Anal. Chem.*; **49:** 464 -469 (1977).
18. Henry, R.G. : Clinical Chemistry . Chemicals and Technique. (2nd Ed), Harper, New york. P.257 (1974).
19. Trinder, P. : Enzymatic method for uric acid determination. Ann. Clin. Biochem., 3(6):29-30 (1969b).
20. Trinder, P. : Enzymatic method for glucose determination. Ann. Clin. Biochem; 3 (6):29-30 (1969a).
21. Woodhead, O.; Otton, P. and Spake, L. : Radioimmunoassay of insulin .Clin.Pharmacol.(21); 11:15 (1974).
22. Snedecor, G.W. and Cochran, W.G. : Statistical Methods (8th Ed), Ames Iowa State University (1982).
23. Gazioano, T.A.; Galea, G., and Reddy, K.S.: Scaling up interventions for chronic disease prevention. *Lancet*; **370:** 1939-1946 (2007).
24. Greenlee, H.; Abasca, K.; Yamerl, E. and Ladas, E.: Clinical applications of Silybum marianum in oncology, Intergrative. *Cancer therapies* **6:** 158-165 (2007).
25. Tamayo, C. and Diamond, S.: Review of clinical trials evaluating safety and efficacy of milk thistle (Silybum marianum «L.» Gaertn). *Integrative Cancer Therapies.* **6:** 146-157 (2007).
26. Szilard, S.; Szentgyorgyi, G. and Dhanalakshmi, S. : Protective effect of Legalon in workers exposed to organic solvents. *Ada Med.* **45:** 249-256 (1988).
27. Gruenwald, J. : Herbal Medicines (3rdEd). Montvale, NJ: Thomson PDR. *Diabetologia,* **18:** 441- 446 (2004).
28. Yadiv, H.; Mukesh, A. and Shalm, Z. : Preventive effect of Rosiglitazone on *Diabetes mellitus. J. Pharmacol Sci.*, **10**: 12-21 (2007).
29. Abbas, A. : *Ph.D. Thesis* (pharmacology) presented to Faculty of Vet. Med., Zagazig University (2009).
30. 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).
31. Satyanarayana, S.A. : Pharmacological activities of *Nigella sativa. Ind. J. Pharm.,* **37:** 126 (1975).
32. El-Dakhakhny, M., Barakat, M., Abd-El-Halim, M., and Aly, S.M. : Effects of Nigella sativa oil on gastric secretion and ethanol induced ulcer in rats. *Journal of Ethnopharmacology.* **72:** 299-304 (2000).
33. Mahmoud, M.R.; El-Abhar, H.S. and Saleh, S. : The effect of Nigella sativa oil against the liver damage induced by Schistosoma mansoni infection in mice. *Journal of Ethnophar-macology.* **79:** 1-11 (2002).
34. Flora, K.; Hahn, M.; Rosen, H. and Benner, K. : Milk thistle (Silybum marianum) for the therapy of liver disease. *Am J Gastroenterol;* **93**(2): 139-43 (1998).
35. Blumenthal, M.: Expanded Commission E Monographs Boston: *Integrative Medicine publications.* **8**: 15-35 (2000).
36. Blumenthal. M. : The ABC Clinical Guide to Herbs .New york : Thieme . PP.285-295 (2003).
37. Jacobs, B.P.; Dennehy, C. and Ramirez, G. : Milk thistle for the treatment of liver disease: a systematic review and meta-analysis. Am J Med. **113**(6): 506-15 (2002).
38. Lieber, C.S.; Leo, M.A.; Cao, Q.; Ren, C. and DeCarli. L.M. : "Silymarin retards the progression of alcohol-induced hepatic fibrosis in baboons". *Journal of Clinical Gastroenterology*. **37**(4): 336-9 (2003).
39. Jones, R.H.; Hayakawa, H. and Mackay, J.D. : Progression of diabetic nephropathy. *Lancet ;* **1:** 1105-1106 (1979).
40. Parving, H.H.; Andersen, A.R. and Smith, U.M. : Effect of antihypertensive treatment on kidney function in diabetic nephropathy. B.M.; **294:** 1443 (1981).
41. Hostetter, T.H.; Troy. J.L. and Brenner, B.M. : Glomerular hemodynamics in experimental diabetes mellitus. *Kidney. Int;* **19:** 401-415

(1981).

42. Ledi, M.; Hohenecker, J. and Francesconi, C. : Acute myopathy in type 2 diabetic patients on combination therapy with Metformin and Rosiglitazone. *Diabetologia,* **48**(10): 1996-1998 (2005).
43. Khan, C.R. and Gordan, G.C. : Joslin's Diabetes Mellitus. (13th ed.) Lea & Febiger. Philadelphia, Baltimore, Hong-Kong, London, Munich, Sydney. Tokyo. A waverly Company (1994).
44. Wagstaff, A.J. and Goa, K.L. : Rosiglitazone: a review of its use in the man-agement of type-2 diabetes mellitus. *Drugs;* **62:** 1805-1837 (2002).
45. Guyton, A.C. and Hall, J.E. : Textbook of Medical Physiology, Endocrinology and Reproduction. Insulin, Glucagon and Diabetes Mellitus. (10th Ed)., W.B. Saunders Company, USA (2000).
46. Yosefy, C.; Magen, E.; Kiselerich, A.; Priluk, R.; London, D.; Volchek, L. and Viskoper, S. : Rosiglitazone improves, while Glibenclamide worsens blood pressure control in treated hypertensive diabetic and dyslipidemic subjects via modulation of insulin resistance and sympathetic activity. *J. Cardiovasc-pharmacol.* **44**(2): 215-222 (2004).
47. Derosa, G.; Gaddr, A.V.; Piccinni, M.N.; Ciccarelli, N. and Salvadeo, S. : Antithrombotic effects of rosiglitazone metformin versus glimepiride-metformin combination therapy in patients with type II diabetes mellitus and metabolic syndrome. *Pharmacotherapy*, **25**(5): 637-645 (2007).
48. Pietruck, E.; Kribben, A. and Vant, A. : Rosiglitazone is a safe and effective treatment option of new onset diabetes mellitus after renal transplantation. *Transpl Int.,* **18**(4): 483-486 (2005).
49. Wagstaff, A.J., and Goa, K.L. : Rosiglitazone: a review of its use in the man-agement of type-2 diabetes mellitus. *Drugs;* **62:** 1805-1837 (2002).
50. Joe, W.S.; Kim, H.j. and Kang. E.S. : The association of total and differential white blood cell counts with metabolic syndrome in type 2 diabetic patients. *Diabetes Research. Clin. Pract;* **73**(3) 284 (2006).
51. Strowing, S.M. and Raskin, K.j. : The effect of rosiglitazone on overweight subjects with type ² diabetes. *Diabetes care,* **28**(7): 1562-1567 (2005).
52. Kao, P.C.; Wu, T.J.; Ho, L.L.T. and Li, X.J. : Review: current and new approaches in the management of diabetes mellitus. *Ann Clin lab Sci.,* **30:** 339-345 (2000).
53. Al-Awadi, F.; Faiania, H., and Shamtc, U. : The effect of a plants mixture extract on liver gluconeogenesis in streptozotocin induced diabetic rats. *Diabetes Research Clinical and Experimental,* **18**, 163-168 (1991).
54. Flaim, K.E.; Huston, S.M.; Lioyd, C.E.; Taylor, J.M.; Shiman, R. and Jefferson, L.S. : Direct effect of insulin on albumin gene expression in primary cultures of rats hepatocytes. *Am. J. Physiol.,* **249:** 447-453 (1985).
55. Benhaddou–Andaloussi, A.; Martineau, L.C.; Spoor, D.; Vuong, T.; Leduc, C.; Joly, E.; Burt, A.; Meddah, B.; Settaf, A.; Arnason, J.T.; Prentki, M. and Haddad, P.S. : Antidiabetic activity of Nigella sativa seed extract in cultured pancreatic â-cella.Skeletal muscle cells and adipocytes; *Pharmaceutical Biology*; **46:** 96-104, (2008).
56. Haddad, P.S.; Dé Pôt, M.; Settaf, A.; Chabil, A. and Cherrah, Y. : Comparative survey on the medicinal plants most recommended by traditional practitioners in Morocco and Canada. *Journal of Herbs Spices and Medicinal plants.* **10:** 25-45 (2003).
57. Eskander, E.R.; Won, J.R.; Ibrahim, K.A., and Abdelal, W. : Hypoglycemic effect of a herbal formulation in alloxan induced diabetic rats, Egypt. *J. Pharm. Sci.* **36**(1 -6), 253-270 (1995).
58. Al-Zuhair, H.H.; El Sayed, M.I., and Sadek, M.A. : Hypoglycemie effect of the volatile oils of Nigella sativa and Allium sativum and their interactions with glipizide on alloxan diabetic rats. *International Journal of Pharmacognosy.* **71:** 85-100 (1996).
59. Wiley, J. and Sons, I. : Herbal Medicine Silymarin may help sugar-control In People with type II diabetes. Science Daily. Retrieved July 22, 2009, (2006). from http://www.sciencedaily.com/releases /2006/10/061030071127.htm