

Impact of Metformin on Immunity and Male Fertility in Rabbits with Alloxan-Induced Diabetes

Naglaa, Z.H. Eleiwa* ; Hesham, A.M.; Hosny Abdel Fadil and Abdel Motal, S.M

Dept. of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Egypt
[*eleiwa02@yahoo.com](mailto:eleiwa02@yahoo.com)

Abstract: A study was designed to explore the possible side effects of metformin on immunity and fertility of male rabbits with alloxan- induced diabetes. Sixteen adult male rabbits were used in this study, they were classified into four equal groups as follows: the first group received neither alloxan nor metformin and remained as control group. Rabbits in the 2nd group were orally treated with metformin at a dose of 120 mg/kg b.wt once a day for 3 months. Rabbits in the 3rd group were administered alloxan, I/V, at a single dose of 100 mg/kg b.wt. Rabbits in the 4th group were administered alloxan (100 mg/kg b.wt, single I/V dose) then treated orally with metformin (120 mg/kg b.wt.) once daily for 3 months. Rabbits in all groups were subcutaneously injected with 2 ml polyvalent rabbit pasteurellosis vaccine after two months from the beginning of experiment for studying the immunological profile of the drug. Treatment of diabetic and non-diabetic rabbits with metformin evoked a significant decrease ($P < 0.05$) in nitric oxide production on the 1st and the 2nd day post vaccination. In response to treatment with metformin, rabbits demonstrated a significant decrease ($P < 0.05$) in serum lysozyme activity on the 1st, 2nd, 3rd day and in the 1st week post vaccination while diabetic rabbits treated with metformin showed a significant decrease ($P < 0.05$) in serum lysozyme activity on the 3rd day and on the 1st, 3rd and 4th week post vaccination. In addition, treatment with metformin of diabetic and non-diabetic rabbits resulted in a significant decrease ($P < 0.05$) in testicular weight, sperm cell count, sperm motility and serum testosterone with a significant increase in sperm abnormalities and dead sperm %. Summing up our observations, the present study calls into question the justification for the use of metformin in a frame of a therapeutic strategy for diabetes due to its resulting negative impact on immunity and male fertility.

[Naglaa, Z.H. Eleiwa; Hesham, A.M.; Hosny Abdel Fadil and Abdel Motal, S.M. Impact of Metformin on Immunity and Male Fertility in Rabbits with Alloxan- Induced Diabetes. Journal of American Science 2010;6(11):417-426]. (ISSN: 1545-1003).

Key words: Metformin – alloxan – diabetes - rabbits

1. Introduction:

Diabetes mellitus is a syndrome characterized by disturbed metabolism and inappropriately high blood sugar (hyperglycaemia) resulting from either low levels of insulin or abnormal resistance to insulin's effects coupled with inadequate levels of insulin secretion to compensate (Tierney et al., 2002).

Treatment of type II diabetes has greatly improved due to the availability of new classes of oral antidiabetic drugs (OADs) and new insulin analogs (Rosak, 2002).

Metformin is one of antidiabetic drugs which belongs to the biguanide class of oral antihyperglycemic agents. It was first synthesized in 1929 and was shown to be a potent hypoglycemic agent, it was rediscovered in 1957 and widely used in Europe to treat type II obese patients. Metformin resurfaced in the 1980s and it was shown to increase insulin sensitivity; this encouraged its introduction to clinical practice in the United States for the first time (Bell and Hadden., 1997)

Metformin acts in the presence of insulin to increase glucose utilization and reduce glucose production, thereby countering insulin resistance. The effects of metformin include increased glucose uptake, oxidation and muscle glycogenesis, increased glucose metabolism to lactate by the intestine, reduced hepatic gluconeogenesis and possibly a reduced rate of intestinal glucose absorption (Clifford, 1993)..

On the other hand, many investigations reported some side effects of metformin therapy as lactic acidosis (Brassøe et al.,2005), vitamin B12-malabsorption that was recorded in about 1/3 of the diabetic cases (Ting et al.,2006) and high incidence of gastrointestinal side effects (Hoffman et al.,2003) as well as higher homocysteine levels(Wulffele et al., 2003).

It is extremely true and fitting to mention that the clinical effectiveness of a given drug would be in jeopardy if its adverse effects outweigh its efficacy. Therefore, the present work was designed to

explore the possible side effects of metformin on immunity and male fertility in rabbits with alloxan-induced diabetes.

2. Materials and methods

Materials:

1-Drug:

Metformin hydrochloride (Glucophage)[®], a product of Minapharm company, Egypt. Metformin is present in the form of tablet containing 1500mg of active drug.

Chemical name:

1,1-Dimethylbiguanide hydrochloride.

2-Alloxan:

Alloxan was purchased from El-Gomhoria Company, Egypt. It was given for induction of diabetes

3-Vaccine:

Formalized polyvalent rabbit pasteurellosis vaccine (VET. SER., VACC. RES. INST.-CAIRO-EGYPT) was used at a dose of 2ml s/c after two months from the beginning of experiment for immunological investigation

4- Experimental animals:

Sixteen adult male rabbits, weighing 2kg each, were obtained from Salsabil company, Fakous, Sharkeya, Egypt and allowed to acclimatize for a week at the animal house at the faculty of Veterinary Medicine. Animals were randomly assigned into four equal groups, four rabbits each and kept in a cage of four separate divisions, maintained at a 12-hour light dark cycle and a constant temperature of $23 \pm 2^{\circ}\text{C}$, received regular rabbit chow (Standard laboratory chow) and water was provided ad-libitum.

Methods:

Induction of diabetes:

Diabetes was induced in two groups of animals (The third and the fourth ones) by single intra-venous injection of alloxan with a concentration of 10% solution in 0.9% NaCl, at the dose of 100 mg/kg. b.wt. Diabetic status was confirmed when the fasting blood sugar value was above 200 mg/dl (Nammi et al., 2003).

Experimental design:

Sixteen adult male rabbits were used in this study, they were kept under hygienic conditions and fed on basic ration free from any medications or chemical additives and water was provided ad-lib.

Rabbits were classified into four equal groups, as follows:

The first group (Control group, Non diabetic non treated.):

Rabbits in the first group received neither alloxan nor metformin and remained as control group.

The second group (Non diabetic treated with metformin):

Rabbits in the second group were treated with metformin at a dose of 120 mg/kg b.wt per os through stomach tube after morning meal once a day for 3 months (Marquie, 1983).

The third group (Diabetic non treated, diabetic control):

Rabbits in the third group were injected I/V with alloxan at a single dose of 100 mg/kg b.wt.

The fourth group (Diabetic treated with metformin):

Rabbits in the fourth group were administered alloxan at a single dose of 100 mg/kg b.wt. (I/V), then treated with metformin at a dose of 120 mg/kg b.wt. Orally. The drug was given to rabbits early in the morning after meal once daily for 3 months

Rabbits in all groups were subcutaneously injected with 2 ml polyvalent rabbit pasteurellosis vaccine after two months from the beginning of experiment for immunological studies.

Laboratory assay:

A- Collection of samples:

5ml of venous blood samples were collected from the ear vein of all rabbits after 24, 48, 72hrs and 1st, 2nd, 3rd and 4th weeks of vaccine administration. The collected blood samples were allowed to clot. Clear serum samples were obtained by centrifugation of blood samples at 3000 rpm for 20 min. One part of serum samples was used immediately for blood glucose level estimation using spectrophotometer by glucose oxidase method (Barham and Trinder, 1972). The other part was stored at -20°C for immunological evaluation and measurement of serum nitric oxide production, lysozyme activity and testosterone level.

At the end of experimental period (3 months), rabbits were slaughtered, testes were dissected and weighed, tails of epididymis were collected for semen analysis.

B- Immunological evaluation:

1-Measurement of serum nitric oxide production:

Nitric oxide level in the serum was measured by spectrophotometer according to Rajaraman et al., (1998).

2-Measurement of lysozyme activity by agarose gel cell lysis assay:

The lysozyme activity in the serum was measured according to Schultz (1987).

The areas under the curve (AUCs), representing lysozyme activity and nitric oxide release through the 30th days post vaccination, were evaluated as shown previously (Abdel Motal et al., 1987). The areas for the control were expressed as 100%, the others relative to it.

C- Male fertility evaluation:

I-Testicular weight:

Was done by using digital electrical balance.

II- Semen analysis:

Was done according to method described by Williams et al., (1990) for examination of both sperm cell concentration, sperm motility, abnormalities and live/dead ratio.

III- Measurement of serum testosterone level:

Serum testosterone levels were estimated according to Burtis and Ashwood, (1994) using active testosterone enzyme immunoassay (EIA) DSL-10-4000 kit obtained from Diagnostic Systems Laboratories Inc.

D- Statistical analysis:

The data were analyzed using SPSS program. Results were reported as mean \pm S.E .The total variations were analyzed by performing the statistical design T-test. Probability levels of less than 0.05 were considered significant (SPSS, WIN.2003).

3. Results

I. Effect of metformin on blood glucose levels

As shown in table (1) ; treatment with metformin revealed a non significant decrease in blood glucose level on the 1st, 2nd and 3rd day post vaccination and a significant decrease ($P < 0.05$) in its level in the 1st, 2nd, 3rd and 4th week post vaccination compared with the control group.

All diabetic rabbits revealed a highly significant increase ($P < 0.01$) in blood glucose levels 24 hour post vaccination and then after compared with the control group while medication of diabetic rabbits with metformin evoked a highly significant decrease ($P < 0.01$) in blood glucose level in all samples collected post vaccination compared with diabetic rabbits.

Table (1): Effect of metformin (120 mg/kg b.wt. per os) on serum glucose level (mg/dl) in non diabetic and alloxan induced diabetic rabbits. (Mean \pm S.E) (n = 4)

Groups	Serum glucose level (mg/dl)						
	Time post vaccination						
	1 st day	2 nd day	3 rd day	1 st week	2 nd week	3 rd week	4 th week
control	108.33 ± 6.56	101.0 ± 0.57	102.66 ± 4.33	98.0 ± 3.00	102.00 ± 4.33	104.0 ± 3.60	101.66 ± 6.38
Treated with metformin	90.33 ± 6.56	88.88 ± 6.02	87.0 ± 4.35	84.66 $\pm 3.17^*$	87.0 $\pm 4.04^*$	85.00 $\pm 3.13^*$	78.66 $\pm 2.96^*$
Diabetic control	443.33 $\pm 20.88^{**}$	426.66 $\pm 8.871^{**}$	414.66 $\pm 18.27^{**}$	415.0 $\pm 13.01^{**}$	411.0 $\pm 17.15^{**}$	417.33 $\pm 12.38^{**}$	425.00 $\pm 11.71^{**}$
Diabetic treated with metformin	175.33 $\pm 20.95^{++}$	162.66 $\pm 15.45^{++}$	188.0 $\pm 5.13^{++}$	175.0 $\pm 2.51^{++}$	182.33 $\pm 2.96^{++}$	180.33 $\pm 20.69^{++}$	166.0 $\pm 21.45^{++}$

* $P \leq 0.05$

** $P \leq 0.01$

* Compared with non diabetic- non treated

+ $P \leq 0.05$

++ $P \leq 0.01$

+ Compared with diabetic- non treated

II. Effect of metformin on the immunological response:

A. Effect on nitric oxide production:

As shown in Fig. (1) and table (2) ; treatment with metformin exhibited a significant decrease ($P < 0.05$) in nitric oxide production on the 1st and 2nd day post vaccination and non significant decrease ($P < 0.05$) in nitric oxide production on the

3rd day, 1st, 2nd, 3rd and 4th weeks post vaccination when compared with control group.

Diabetic rabbits showed a highly significant decrease ($P < 0.01$) in nitric oxide production on the 1st and 2nd day post vaccination and non significant decrease in nitric oxide production on the 3rd day, 1st, 2nd, 3rd and 4th weeks post vaccination compared with the control group.

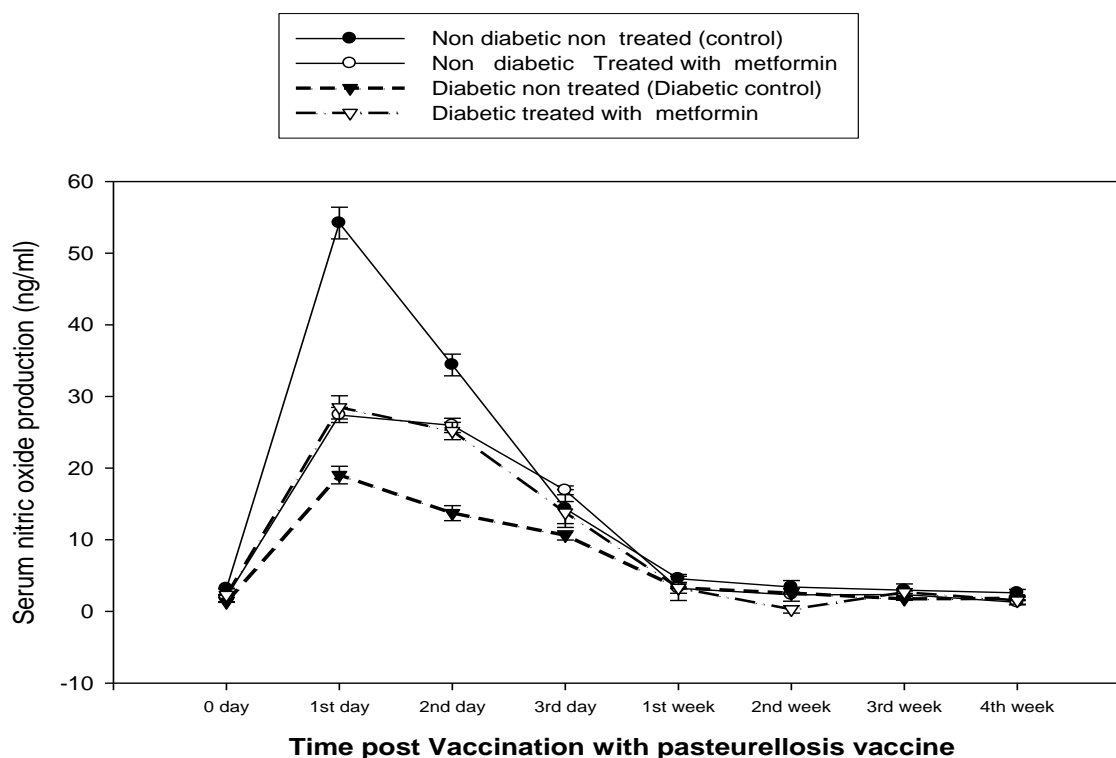


Fig. (1): Effect of metformin (120 mg/kg b.wt. per os) on nitric oxide production (ng/ml) in non diabetic and alloxan induced diabetic rabbits.

Treatment of diabetic rabbits with metformin demonstrated a significant decrease in nitric oxide production on the 1st and the 2nd day post vaccination and non significant decrease in nitric oxide production on the 3rd day, 1st week, 2nd week, 3rd week and 4th week post vaccination compared with the control group.

Rabbits treated with metformin displayed 27.27% decrease in nitric oxide release all over the 30th days post vaccination. Diabetic rabbits demonstrated 45.45% decline, diabetic rabbits treated with metformin showed 27.27% decrement.

B. Effect of metformin on serum lysozyme activity.

As shown in Fig. (2) and table (2); treatment with metformin demonstrated a significant decrease ($P < 0.05$) in serum lysozyme activity on the 1st, 2nd and a highly significant decrease ($p < 0.01$) on the 3rd day and in the 1st week post vaccination and non significant decrease in serum lysozyme activity in the 2nd, 3rd and 4th week post vaccination compared with the control group.

Table (2): Effect of metformin (120 mg/kg b.wt. per os) given on nitric oxide production and lysozyme activity (represented by AUC and expressed as %) in rabbits with alloxan - induced diabetes.

GROUPS	NITRIC OXIDE (%)	Lysozyme (%)
Control	100	100
Treated with metformin	72.73	80
Diabetic control	54.55	66.66
Diabetic treated with metformin	72.73	80

Diabetic rabbits revealed a significant decrease ($P < 0.05$) in serum lysozyme activity on the 1st, 2nd, 3rd and 4th week and a highly significant decrease ($p < 0.01$) on the 3rd day and in the 1st week post vaccination compared with the control group.

Diabetic rabbits treated with metformin showed non significant decrease ($P < 0.05$) in serum lysozyme activity on the 1st day, 2nd day and 2nd week post vaccination and a significant decrease in serum lysozyme activity on the 3rd day and on the 1st, 3rd and 4th week post vaccination compared with the control group.

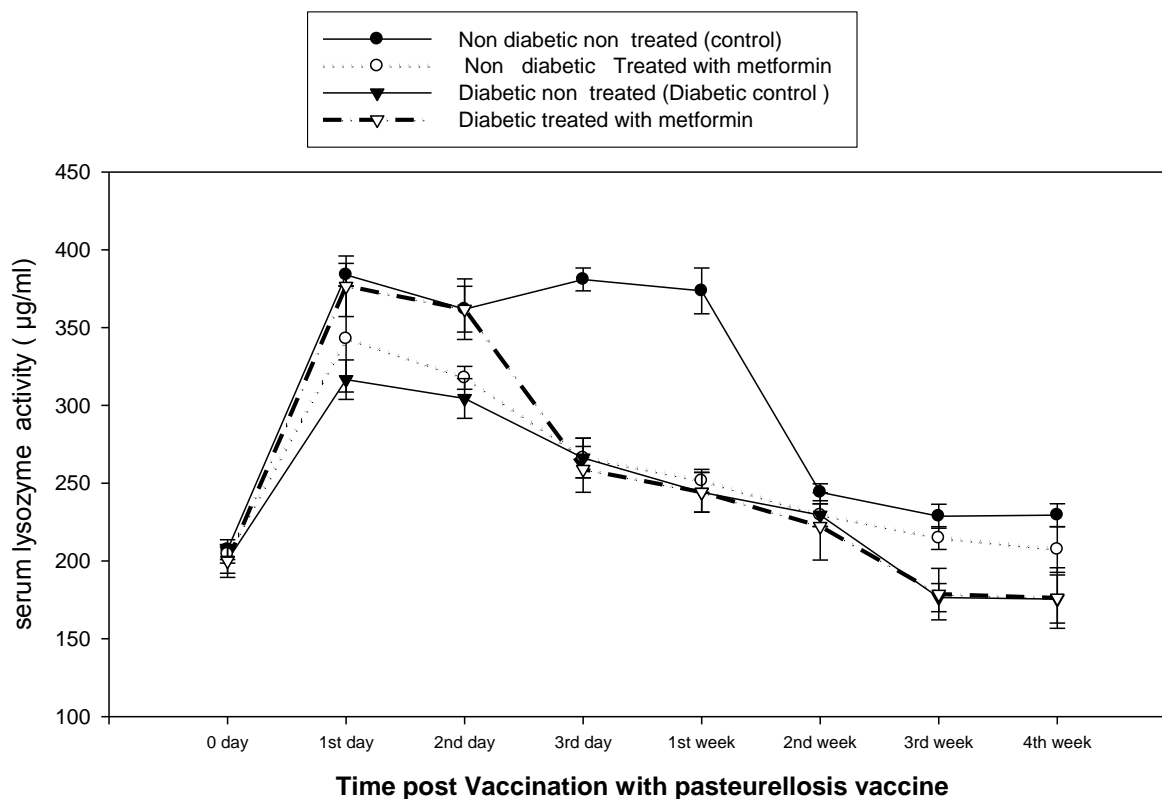


Fig. (2): Effect of metformin (120 mg/kg b.wt. per os) on serum lysozyme activity (µg/ml) in non diabetic and alloxan induced diabetic rabbits.

During the 30th days post challenge with polyvalent rabbit pasteurellosis vaccine, rabbits treated with metformin displayed 20% decrease in lysozyme activity. Diabetic rabbits showed 33.34% decline, diabetic rabbits treated with metformin displayed 20% decrease.

III. Effect of metformin on male rabbit fertility:

1) Effect on testicular weight

In response to treatment with metformin, rabbits demonstrated a significant decrease ($P < 0.05$) in testicular weight compared with the control group, while, diabetic rabbits exhibited non significant decrease in testicular weight compared with the control group (table 3).

Treatment of diabetic rabbits with metformin evoked a highly significant decrease ($P < 0.01$) in testicular weight compared with the diabetic rabbits.

2) Semen analysis

a) Effect on sperm cell count

Treatment of rabbits with metformin showed a highly significant decrease ($P < 0.01$) in sperm cell count compared with the control group.

Diabetic rabbits demonstrated a significant decrease in sperm cell count compared with the control group.

Medication of diabetic rabbits with metformin elicited a highly significant decrease ($P < 0.01$) in sperm cell count compared with the diabetic rabbits.

b) Effect on sperm motility

Treatment of rabbits with metformin, displayed a significant decrease in sperm motility compared with the control group.

Diabetic rabbits displayed non significant decrease in sperm motility compared with the control group.

Treatment of diabetic rabbits with metformin induced a highly significant decrease ($P < 0.01$) in sperm motility compared with the diabetic rabbits.

Table (3): Effect of metformin (120 mg/kg b.wt. per os) on male fertility in non diabetic and alloxan induced diabetic rabbits. (Mean + SE) (n = 4)

Parameters Groups	Testicular weight (gm)	Sperm cell count (sp.c.c x 10 ⁶ /ml)	Sperm motility (%)	Dead (%)	Sperm abnormalities (%)	Serum testosterone Level (ng/ml)
control	20.33± 1.45	160.83± 5.83	91.66± 1.66	7.00± 0.28	9.56± 0.34	0.58±0.30
Treated with metformin	15.33± 0.33*	110.00± 1.44**	80.00± 2.88*	13.26± 0.43**	13.50± 0.76**	0.11±0.01*
Diabetic control	17.66± 0.33	135.83± 5.83*	88.33± 1.66	12.33± 0.88**	15.76± 0.39**	0.13±0.03*
Diabetic treated with metformin	9.33± 0.88++	17.33± 1.45++	55.33± 2.88++	14.40± 0.30	24.16± 0.72++	0.08±0.00+

* P ≤ 0.05

** P ≤ 0.01

* Compared with non diabetic- non treated

+ P ≤ 0.05

++ P ≤ 0.01

+ Compared with diabetic- non treat

c) Effect on dead sperm%

Rabbits treated with metformin exhibited a highly significant increase (P<0.01) in dead sperm % compared with the control group.

Diabetic rabbits demonstrated a highly significant increase (P<0.01) in dead % compared with the control group.

Medication of diabetic rabbits with metformin showed non significant increase in dead % compared with the diabetic rabbits.

d) Effect on sperm abnormalities

In response to treatment of normal rabbits with metformin, rabbits demonstrated a highly significant increase (P<0.01) in sperm abnormalities (coiled tail sperm and detached tail sperm) compared with the control group.

Diabetic rabbits displayed a highly significant increase (P<0.01) in sperm abnormalities (detached tail sperm and stunted growth sperm) compared with the control group.

Treatment of diabetic rabbits with metformin induced a highly significant increase (P<0.01) in sperm abnormalities (coiled tail sperm and detached head and tail sperm) compared with the diabetic rabbits.

3) Effect on serum testosterone level

Rabbits treated with metformin displayed a significant decrease (P<0.05) in serum testosterone compared with the control group.

Diabetic rabbits demonstrated a significant decrease (P<0.05) in serum testosterone compared with the control group.

Medication of diabetic rabbits with metformin revealed a significant decrease (P<0.05) in serum testosterone compared with the diabetic rabbits.

4. Discussion:

Metformin (dimethylbiguanide) is an antihyperglycaemic drug used to treat non-insulin dependent diabetes mellitus. It acts in the presence of insulin to increase glucose utilization and reduce glucose production, thereby countering insulin resistance. The effects of metformin include increased glucose uptake, oxidation and muscle glycogenesis, increased glucose metabolism to lactate by the intestine, reduced hepatic gluconeogenesis and possibly a reduced rate of intestinal glucose absorption. Metformin appears to facilitate steps in the post receptor pathways of insulin action, and may exert effects that are independent of insulin. In muscles, metformin increases translocation into the plasma membrane of certain isoforms of the glucose transporter (Clifford, 1993).

Nitric oxide (NO), has a wide variety of effects on cells. It has been found to affect different kinds of cells, with particularly striking effects in the control of blood pressure and the immune system (Rang et al., 1999).

In all sites, where NO is active, it is produced by the reaction of arginine with molecular oxygen to give citrulline as well as NO. The reaction is catalyzed by the enzyme nitric oxide synthase (NOS). NOS exist in several slightly different forms, depending on the kind of cell in which is found (Campbell, 1995).

Acknowledging the differences, it could be reasoned that a similar effect of NOS in the immune system might pertain.

In the current study, healthy rabbits treated with metformin demonstrated a significant decrease in nitric oxide production 24 and 48 hour post vaccination compared with the control.

Regrettably enough, our data are by no means sufficient to provide us with a ready clarification for the previous effect.

Nevertheless, going through literature could provide us with all right clue.

Convincing evidence is accumulating for the ability of vitamin B12 to modulate cellular immunity especially lymphocyte counts, CD8 cells and the natural killer (NK) cells. (Tamura et al., 1999).

Similarly, Erkurt et al., (2008) reported that vitamin B12 had important immunomodulatory effects on cellular immunity, and abnormalities in the immune system in pernicious anemia are restored by vitamin B(12) replacement therapy.

In vitamin B12-deficient patients, numbers of CD4 and CD8 lymphocytes decreased, CD4/CD8 ratio increased, and NK cell activity was depressed. After cyanocobalamin treatment, absolute numbers and percentage of lymphocyte subgroups were elevated. Increased CD4/CD8 ratio and depressed natural killer (NK) cell activity were restored and levels of C3, C4, and immunoglobulins were elevated (Erkurt et al., 2008).

The use of a multinutrient containing optimum amounts of essential trace elements and vitamins (as beta-carotene, Vitamins B6, B12, C, D and E, and folic acid) result in enhanced immune responses and reduction in the occurrence of common infections (Chandra, 2004).

Vitamins (A, B6, B12, C, D, E and folic acid) and the trace elements (iron, zinc, copper and selenium) work in synergy to support the protective activities of the immune cells. All these micronutrients are essential for antibody production. Overall, inadequate intake and status of these vitamins and trace elements may lead to suppressed immunity, which predisposes to infections and aggravates malnutrition. Thus, supplementation with these selected micronutrients can support the body's natural defense system by enhancing all three levels of immunity (physical barriers (skin/mucosa), cellular immunity and antibody production) (Maggini et al., 2007).

Kaplan and Basford (1976) noted that morphological and quantitative neutrophil abnormalities are common in the megaloblastic anemias of vitamin B12 and folic acid deficiency. The authors recorded that vitamin B12 has specific role in the production of intermediates necessary for normal cell function.

Patients receiving metformin have diminished vitamin B12 (Bauman et al., 2000 and Ting et al., 2006). Sahin et al., (2007) reported that patients with type 2 diabetes, metformin reduced levels of folate and vitamin B12 and increases homocysteine (Hcy). This deficiency was occurred through interfering with its absorption in the distal ileum (Quillen et al., 1999 and Rufenacht et al., 2008).

Given the previous tapestry, it might be tenable to point out that vitamin B12 deficiency induced by metformin could account for the significant decrease in NO production.

In the current study, alloxan-induced diabetes elicited a significant decrease in serum lysozyme activity 24 and 48 hour and in the 3rd and 4th week post vaccination.

Lysozyme is bactericidal for almost all bacteria, hydrolyzing its cell wall resulting in its lysis. It activates the complement system and phagocytes (Jolles and Jolles, 1984).

The lysozyme protein (N-acetylmuramidase) is a major component of the specific granules of the polymorphonuclear leucocytes (PMN) and is found in high concentrations in the mucosal secretions in the eyes, oropharynx, respiratory tract and vagina. Several lines of evidence suggest that its localization at these sites is related to its role in the defense system. It is actively secreted by (PMN) cells during inflammatory response into the external environment as it has antimicrobial activity by degrading the glycosidic linkage of bacterial peptidoglycan (Richard and Theodore, 1991).

Hyperglycemic environment may enhance the virulence of various microorganisms. *Candida albicans* shows competitive binding and inhibition of complement mediated phagocytosis in a hyperglycemic environment (Hostetter, 1990).

Glucosuria enhances *Escherichia coli* growth and may be a reason for the increased incidence of urinary tract infections in diabetics (Geerlings et al., 1999).

Oxidative stress which is the metabolic response to hyperglycemia in patients with DM, may affect neutrophil lifespan, and phagocytic cell function resulting in a decrease in their ability to prevent or eliminate infection (Geerlings and Hoepelman, 1999 and Watson, 2002).

In general, patients with diabetes are at high risk of infections, which are more serious and prolonged. It is notable that the circulating levels of proinflammatory cytokines are elevated in diabetic patients and it has been suggested that the impaired functions of neutrophils contribute to the increased susceptibility to infections observed in these patients (Pickup et al., 2000).

The neutrophils of diabetic patients display increased necrosis and enhanced production of reactive oxygen species (Shurtz-Swirski et al., 2001). In the present experiment, healthy rabbits treated with metformin showed a significant decrease in serum lysozyme activity 24 and 48 hour post vaccination.

As pointed out previously with nitric oxide finding, the reduction in lysozyme activity, another

representative of cellular immunity could be further rationalized by vitamin B12 deficiency.

In the present study, alloxan induced diabetes in rabbits elicited a significant decline in sperm cell count and serum testosterone, and increase in dead % and sperm abnormalities.

In like manner, Naziroğlu, (2003) reported that streptozotocin (STZ) induced diabetes in rats was associated with impairment of testicular function leading to reduced fertility. Its etiology may involve oxidative damage by reactive oxygen species, and protection against this damage can be offered by antioxidant supplementation.

Similarly, Scarano et al. (2006) recorded that diabetes resulted in decreased body and reproductive organ weights, as well as diminished sperm counts in the testis and epididymis, associated with diminution of plasmatic testosterone levels, after natural mating, there was a decrease in the fertility in the diabetic adult male rats (25.5%) compared with control animals (81.5%).

Likewise, Shrilatha and Muralidhara, (2007) observed oxidative impairments in testis of STZ-induced diabetes in adult rats developed over time might at least in part contribute towards the development of testicular dysfunction through testicular degeneration leading to reduced fertility. Interestingly enough, diabetic rabbits medicated with metformin revealed a significant decrease in testicular weight, sperm cell count, sperm motility and serum testosterone, as well as an increase in dead % and sperm abnormalities.

Given the evidence, one is intrigued to surmise that the reproductive dysfunction seen in the shade of metformin treatment may be imputed to vitamin B12 deficiency.

The previous contention is commendable by the notion that vitamin B12 deficiency is induced by long-term use of metformin (Lin et al., 2007)

In this frame of reference, it is interesting to note that Vitamin B₁₂ deficiencies in men can lead to reduced sperm counts and lowered sperm motility. For this reason, B₁₂ supplements have been tried for improving fertility in men with abnormal sperm production (Kumamoto et al., 1988).

The concept is further boosted by the fact that mecobalamin (Me-B 12) enhances testicular function, resulting in an increased output of mature sperm. By oral administration of Me B 12 (1.0 mg/kg/day) to the oligozoospermic mice for 10 weeks, the sperm count, sperm motility, motile sperm count, diameter of seminiferous tubules and the percentage of good motile sperm were increased as compared with those of the control (Oshio et al., 1989).

On similar grounds, Sinclair, (2000) noted that number of nutritional therapies have been shown to improve sperm counts and sperm motility, including carnitine, arginine, zinc, selenium, and vitamin B12.

5. Conclusion:

Summing up our observations, the present study calls into question the justification for the use of metformin in a frame of a therapeutic strategy for diabetes due to its resulting negative impact on immunity and male fertility.

Corresponding author

Naglaa ,Z.H. Eleiwa
Dept. of Pharmacology, Faculty of Veterinary
Medicine, Zagazig University, Egypt.
eleiwa02@yahoo.com

6. References:

- 1- Abdel Motal,S.M. ; May,C.M.;Pian-Swith and Geoffery,W.G.Sharp (1987): Effects of tetracaine on insulin release and calcium handling in rat pancreatic islets. *Am.J.Physiol.*252 (Endocrinol.Metab.15): E 727-733.
- 2- Barham, D.and Trinder, P. (1972) An improved color reagent for the determination of blood glucose by the oxidase system.*Analyst.*142-5.
- 3- Bauman, W.A.; Shaw, S.; Jayatilleke, E.; Spungen, A.M. and Herbert, V.(2000):Increased intake of calcium reverses vitaminB12 malabsorption induced by metformin. *Diabetes Care.*; 23(9):1227-31.
- 4- Bell, P.M. and Hadden, D.R.(1997) : Metformin. *Endocrinol Metab Clin North Am.* 26(3):523-37.
- 5- Brassøe, R.; Elkmann, T.; Hempel, M. and Gravholt, C.H. (2005): Fulminant lactic acidosis in two patients with Type 2 diabetes treated with metformin *Diabet Med.* 22(10): 1451-3.
- 6- Burtis, C.A. and Ashwood, F.R. (1994): Tietz Text book of clinical chemistry, 2nd ed. WB Saunders Co., Philadelphia. P 1851.
- 7- Campbell, N.K. (1995): *Biochemistry*2nd ed. Saunders College Publishing.
- 8- Chandra, R.K. (2004): Impact of nutritional status and nutrient supplements on immune responses and incidence of infection in older individuals. *Ageing Res Rev.* 3(1):91-104.
- 9- Clifford, J.Bailey. (1993): *Metformin.General Pharmacology: The Vascular System Vol;* 24: 1299-1309.
- 10-Erkurt, M.A.; Aydogdu, I.; Dikilitaş, M.; Kuku, I. Kaya, E. Bayraktar, N. Ozhan, O.; Ozkan, I. and Sonmez, A. (2008): Effects of cyanocobalamin on immunity in patients with pernicious anemia. *Med Princ Pract.* 17(2):131-5.

- 11-Geerlings, S.E. and Hoepelman, A.I. (1999): Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunol Med Microbiol.* 26: 25965.
- 12-Geerlings, S.E.; Brouwer, E.C. and Gastra W (1999): Effect of glucose and pH on uropathogenic and nonuropathogenic *Escherichia coli*: studies with urine from diabetic and non-diabetic individuals. *Journal of Medical Microbiology.* 48: 535–539.
- 13-Hoffmann, I.S.; Roa, M.; Torrico, F. and Cubeddu, L.X.(2003): Ondansetron and metformin-induced gastrointestinal side effects. *Am J Ther.*;10(6):447-51
- 14-Hostetter, M.K. (1990): Handicaps to host defense. Effects of hyperglycemia on C3 and *Candida albicans*. *Diabetes*; 39: 271–275.
- 15-Jolles, P. and Jolles, J. (1984): What is new in lysozyme research. Always a model system. Today is like yesterday. *Mol. Cell. Biochem.* 63:165-189.
- 16-Kaplan, S.S. and Basford, R.E. (1976): Effect of vitamin B12 and folic acid deficiencies on neutrophil function. *Blood.* 47(5): 801-5.
- 17-Kumamoto, Y.; Maruta, H. and Ishigami J (1988) : Clinical efficacy of mecobalamin in treatment of oligozoospermia—results of double-blind comparative clinical study . *Hinyokika Kiyo.* 34:1109-1132.
- 18-Lin, H.Y.; Chung, C.Y.; Chang, C.S.; Wang, M.L.; Lin, J.S. and Shen, M.C. (2007): Hyperhomocysteinemia, deep vein thrombosis and vitamin B12 deficiency in a metformin-treated diabetic patient. *J Formos Med Assoc.* 106 (9): 774-8.
- 19-Maggini, S.; Wintergerst, E.S.; Beveridge, S. and Hornig, D.H. (2007): Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. *Br J Nutr.* 98 Suppl 1:S29-35.
- 20-Marquié, G.(1983): Metformin action on lipid metabolism in lesions of experimental aortic atherosclerosis of rabbits *Atherosclerosis* ;47(1):7-17.metformin. *Arch Intern Med.* 166(18):1975-9 .
- 21-Nammi, S.; Boini M.K.; Lodagala, S.D. and Behara, R.B.(2003): The juice of fresh leaves of *Catharanthus roseus* Linn. reduces blood glucose in normal and alloxan diabetic rabbits. *BMC Complement Altern Med.* 2: 3:4.
- 22-Naziroğlu, M.(2003): Enhanced testicular antioxidant capacity in streptozotocin-induced diabetic rats: protective role of vitamins C and E and selenium. *Biol Trace Elem Res.* 94(1):61-72.
- 23-Oshio, S.; Ozaki, S.; Ohkawa, I.; Tajima, T.; Kaneko, S. and Mohri, H. (1989) : Mecobalamin promotes mouse sperm maturation. *Andrologia.* 21(2):167-73.
- 24-Pickup, J.C.; Chusney, G.D.; Thomas, S.M. and Burt, D. (2000): Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci* 67:291–300.
- 25-Quillen, DM. Samraj, G. and Kuritzky, L. (1999): Improving management of type 2 diabetes mellitus: Biguanides. *Hosp Pract (Off Ed).* 34:41–44.
- 26-Rajaraman, V.; Nonneck, B. J.; Franklin, S.T.; Hamell, D.C. and Horst, R.L. (1998): Effect of vitamins A and E on nitric oxide production by blood mononuclear leukocytes from neonatal calves fed milk replacer. *J.Dairy Science.* 81:3278-3285.
- 27-Rang, H. P.; Dale, M. M. and Ritter, J. M.(1999) : *Pharmacology*, Fourth edition. (11)188-197.
- 28-Richard, T.E. and Theodore, J.G.(1991): Killing of gram-negative bacteria by lactoferrin and lysozyme. *J. Clin. Invest.*(88)1080-1091.
- 29-Rosak, C. (2002): The pathophysiologic basis of efficacy and clinical experience with the new oral antidiabetic agents. *J Diabetes Complications.* 16(1):123-32.
- 30-Rufenacht, P.; Mach-Pascual, S. and Iten, A. (2008): Vitamin B12 deficiency: challenging diagnosis and a secret treatment *Rev Med Suisse.* 4(175):2212-4, 2216-7.
- 31-Sahin, M.; Tutuncu, N.B.; Ertugrul, D.; Tanaci, N. and Guvener, N.D, (2007): Effects of metformin or rosiglitazone on serum concentrations of homocysteine, folate, and vitamin B12 in patients with type 2 diabetes mellitus. *J Diabetes Complications.* 21(2):118-23.
- 32-Scarano, W.R.; Messias, A.G.; Oliva, S.U.; Klinefelter, G.R. and Kempinas, W.G. (2006): Sexual behaviour, sperm quantity and quality after short-term streptozotocin-induced hyperglycaemia in rats. *Int J Androl.* 29(4):482-8.
- 33-Schultz, L.A. (1987): *Methods in clinical chemistry.* The C.V Mosby Cost Lauris: 742-746.
- 34-Shrilatha, B. and Muralidhara (2007): Occurrence of oxidative impairments, response of antioxidant defences and associated biochemical perturbations in male reproductive milieu in the Streptozotocin-diabetic rat. *Int J Androl.* Dec; 30(6):508-18.
- 35-Shurtz-Swirski, R.; Sela, S. and Herskovits, A.T. (2001): Involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients. *Diabetes Care.* 24:104–10.

- 36-Sinclair, S. (2000): Male infertility: nutritional and environmental considerations. *Altern Med Rev.* 5(1):28-38.
- 37-SPSS Win.(1995): Statistical Program Under Windows, U.S.A.
- 38-Tamura, J.; Kubota, K.; Murakami, H.; Sawamura, M.; Matsushima, T.; Tamura, T.; Saitoh, T.; Kurabayashi, H. and Naruse, T. (1999): Immunomodulation by vitamin B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. *Clin Exp Immunol.* 116(1):28-32.
- 39-Tierney, L. M.; McPhee, S. J. and Papadakis, M. A. (2002): *Current medical Diagnosis & Treatment.* International edition. New York: Lange Medical Books/McGraw-Hill, 1203-1215.
- 40-Ting, R.Z.; Szeto, C.C.; Chan, M.H.; Ma, K.K. and Chow, K.M. (2006): Risk factors of vitamin B12 deficiency in patients receiving metformin. *Arch Intern Med.* 166(18):1975-9.
- 41-Watson, R.W. (2002): Redox regulation of neutrophil apoptosis. *Antiox Redox Signal* 4: 97e104.
- 42-Williams, J., Gladen, B., Schrader, S., Turner, T., Phelps, J. and Chapin, R. (1990): Semen analysis and fertility assessment in rabbits: statistical power and design consideration for toxicology studies: *Fundamentals of applied toxicology*, 15:651-665.
- 43-Wulfele, M.G.; Kooy, A.; Lehert, P.; Bets, D.; Ogterop, J.C.; Borger van der Burg, B.; Donker, A.J. and Stehouwer, C.D. (2003) : Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus *J Intern Med.* 254 (5): 455-63.

9/25/2010