ORIGINAL ARTICLE **Mirror of Research in Veterinary Sciences and Animals MRVSA/ Open Access DOAJ**

Effects of metformin treatment on Iron, Zinc and Copper status concentration in the serum of female rats with induced polycystic ovary syndrome

Muhsin S. G. Al-Moziel[1], Jassim A. A. Alkalby[2], Alaa A. Sawad*[3]

[1] Department of Physiology and Pharmacology, College of Pharmacy, [2] Department of Physiology and Pharmacology, College of Veterinary Medicine; [3] Department of Histology and Anatomy / College of Veterinary Medicine / University of Basra

ARTICLE INFO
Received: 27.04.2013
Revised: 19. 05.2013
Accepted: 25.05.2013
Publish online: 30. 05.2013

***Corresponding author:**
Email address:
alaasawad24@gmail.com

Abstract

This study conducted to investigate the effects of metformin drug on serum Iron, Zinc and Copper concentration in Estradiol Valerate(EV) induced polycystic ovary syndrome(PCOS) in virgin rats. Thirty virgin rats were randomly allotted to constitute Normal control (NC-I) group and induced polycystic ovary (PCO-I and PCO-II) groups having 10 rats in each group. Rats from NC-I group were administered intramuscularly with 0.2 ml of corn oil whereas polycystic ovary was induced in rats from PCO-I and PCO-II groups by administering single intra-muscular injection of estradiol Valerate 4mg/rat. The rats from PCO-I and PCO-II groups were left for 60 days for development of polycystic ovary syndrome. Animals from PCO-I group were then administered with 0.2 ml normal saline as oral gavage for 15 days, these animals were kept as PCO control group animals whereas those from PCO-II groups received metformin (50mg/kg B.wt) as oral gavage for 15 days, these animals served as metformin treated PCO group animals. All the rats were thereafter sacrificed for collecting blood from inferior vena-cava. Serum samples from each rat were assessed for iron, zinc and copper status in each experimental group. The results revealed a significant ($p \leq 0.05$) increase in serum Fe and Zn and a significant ($p \leq 0.05$) decrease in serum Cu concentration in PCO group 1 compared with control non-treated group. The PCO group2 treated with metformin showed a significant ($p \leq 0.05$) decrease in serum Fe concentration as compared with those in animals from group NC-I and PCO-I. While, no significant differences were found in serum Zn concentration between all treated groups. On the other hand, a significant ($p \leq 0.05$) increase in serum Cu concentration appeared in metformin treated group compared with PCO group 1 which appears significant decrease compared with control group.

To cite this article: Muhsin S. G. Al-Moziel., Jassim A. A. Alkalby., Alaa A. Sawad (2013). Effects of metformin treatment on iron, Zinc and Copper status concentration in the serum of female rats with induced polycystic ovary syndrome. Mirror of Research in Veterinary Sciences and animals. MRVSA 2 (2), 54-60.
DOI: 10.22428/mrvsa. 2307-8073.2013. 00228.x

Keywords: *Salmonella mbandaka*, histopathology, *Salmonella* infection.

Introduction

Polycystic ovary syndrome (PCOS), a complex, multifactorial endocrinopathy of reproductive aged women, is a multifactorial reproductive endocrinopathy of aged women. It is the most common cause of infertility (Jacqueline *et al.*, 2012). PCOS is a metabolic disorder associated with insulin resistance attributable to ovarian hyperinsulinemia, however, in women with PCOS metformin treatment restores the cyclic nature of menstruation and increases ovulation (Vandemolen *et al.*, 2001). Metformin is a biguanide currently used as oral antihyperglycemic agent, in additionally, Metformin is an anti-diabetic drug commonly used to treat cycle disorders and anovulation in women with PCOS (Stefano *et al.*, 2009). Multiple concomitant therapies have been applied in PCOS, for such a syndrome is used of Insulin Sensitizer Drugs (ISDs) and drugs lowering androgen secretion, they were later associated with beneficial effects in the treatment of PCOS (Mandakini, 2005).
Metformin has been shown to significantly reduce basal hepatic glucose production, increased peripheral glucose disposal and reduced intestinal absorption (Ripudaman, and Silvio, 2003).
The essential or beneficial effect of many trace elements has been established mainly in laboratory or farm animals rather than in humans, the most important of them are Iron, Zinc and Copper. These trace elements are necessary for the growth and function of the brain where deficiency or excess of these elements resulted in nervous disorders (Takeda, 2004). Many researches have indicated that serum body iron are elevated in patients with PCOS (Angeles *et al.*, 2009; Manuel *et al.*, 2011). Other authors opined that metabolic disorder including hyperinsulinemia is probable cause of iron accumulation in PCOS patients (Faranak *et al.*, 2011; Saeed *et al.*, 2011).
A recent report described reduced levels of Zn in obese and insulin resistance subject (Chausmer, 1998). Whereas, other authors have recorded increase in the serum Zn concentration in diabetics (Vikkorinova *et al.*, 2009). Interestingly it was reported that diabetics have elevated levels of copper (Obeid, et al, 2008) and it could be that copper is in fact linked to metabolic syndrome and diabetes (Wijesekara *et al.*, 2009). Copper is an essential trace element, capable of fluctuating between the oxidized Cu^{2+} and the reduced Cu^{+} state, being co-factor for many enzymes. More, the deficiencies and the excess of Cu are associated with specific clinical manifestations (Guojun *et al.*, 2004).
This study was designed to evaluate effects of metformin treatment on iron, Zinc and Copper status concentration in the serum of female rats with induced polycystic ovary syndrome.

Materials and methods

Thirty virgin adult cycling female rats (200±15 g B.wt.) were housed (4 rats/cage) under optimum identical conditions (12/12 light, dark cycle, 22 ± 2 C˚) wherein these are allowed free access to pelleted rat chow and tap water. Animals showing at least four regulars 4-day cycles were randomly allotted to constitute Normal control (NC-I) group and induced polycystic ovary (PCO-I and PCO-II) groups having 10 rats in each group. Rats from NC-I group were administered intramuscularly with 0.2 ml of

corn oil whereas polycystic ovary was induced in rats from PCO-I and PCO-II groups by administering single intra-muscular injection of estradiol Valerate 4mg/rat. The rats from PCO-I and PCO-II groups were left for 60 days for development of polycystic ovary syndrome. Animals from PCO-I group were then administered with 0.2 ml normal saline as oral gavage for 15 days, these animals were kept as PCO control group animals whereas, those from PCO-II groups received metformin (50mg/kg B.wt.) as oral gavage for 15 days, these animals served as metformin treated PCO group animals. All the rats were thereafter sacrificed for collecting blood from inferior vena-cava. Serum was separated by centrifugation 5000 rpm and stored at -20 C for assessing Fe, Zn and Cu concentration in serum.
This study was approved by research and ethical committee in College of Veterinary Medicine/ University of Basra, no: 25/11/2011.

Determination of serum Fe, Zn and CU Levels

The serum samples from each group were subjected to wet digestion for releasing iron, zinc and copper from the protein matrix (Akinloye *et al.*, 2011), these trace elements were then evaluated in serum by atomic absorption spectrophotometer (AAS) using a Buck Model 211-VGP spectrophotometer according to operator's manual (February, 2005 VER 3.94 C by Analyst: Gerald J. De Menna), with a detection limit of 0.05 ppm for Fe (Akinloye *et al.,* 2009).

Biostatistical analysis

The data were expressed as mean ±Standard Deviation (SD) and analyzed using two way analysis of variance (ANOVA). Least significant difference (LSD) was used to test the differences among means for ANOVA indicated a significant ($P<0.05$), using computerized SPSS version 11.

Results

From different groups (Table-1) reveals significantly ($P\leq0.05$) increased serum iron levels in the rats from induced Polycystic ovary (PCO-I and II) groups as compared to that in normal control (NC-I) group; among PCO groups it was significantly higher in untreated animals (PCO-I group) as compared to that in metformin treated animals (PCO-II group). Serum Zn levels in animals from normal control (NC-I) group was significantly higher than that in animals from untreated polycystic ovary (PCO-I) group, however, serum Zn levels in animals from metformin treated (PCO-II) group did not differ significantly from that in animals from normal control (NC-I) and untreated polycystic (PCO-II) groups. Serum Cu level in animals from normal control group (NC-I) was significantly ($P\leq0.05$) higher than that in animals from metformin compared with control group and PCO group.

Discussion

In the present study, substantial evidence was provide support the increase in Fe, Zn serum concentration associated with decreased Cu concentration in female rats suffering from induced PCO compared with control (table 1). Pervious study was mentioned that insulin resistance plays a major role on the increased body Fe stores of rats with PCOS. On the other hand, the reduced menstrual losses and /or oligomenorrhea may contribute to increased Fe concentration (Mandakini, 2005). Guojun, *et al.,* (2004) explained that patients with PCOS could have led to increase iron concentration. The result of this study is compatible with the other researcher who reported that patients with PCOS showed decreased circulating hepcidin levels and increase ferritin to hepcidin
molar ratios compared with control (Manuel *et al.,* 2011). The results of this study is in agreement with (Gőzdemir *et al.,* 2013) they explained that the hepcidin plays a role in the regulation of metabolism and acts as an inflammatory marker in polycystic ovary syndrome.

Table (1): The effect of treatments for 15 days with metformin on some trace elements concentration in female rats with induced polycystic ovary syndrome.
(Mean ±SD, n=10).

Parameter Groups	Fe/mcg/ml	Zn/ppm	Cu/ppm
Control	b 656.25±11.79	b 6.92±0.25	a 26.61±1.36
PCO 1	a 697.00±7.43	a 8.18±0.23	c 15.24±0.77
PCO2	c 256.25±3.75	ab 7.50±0.29	b 20.25±0.64
LSD Least significant difference	38.12	1.02	5.01

Values expressed in the, b, c; mean significant differences at the (P≤0.05) level.

Moreover, Zn is one of the most important trace elements required as catalytic, structure and regulatory ion for the activities of more than 300 enzyme proteins transcriptional factors (Pouteymour *et al.*, 2011). Current study, revealed increase in the serum Zn concentration in PCO group, this may be associated with disturbance of glucose metabolism represented by insulin resistance, dyslipidemia and endocrine disturbance leading to oxidative stress and finally increase in Fe, and Zn. Mounting evidence indicates that higher body Fe, Zn stores are associated with increased risk of other insulin resistant disorder such as high blood glucose and lipid profile (Yves *et al.,* 2011).

Table (1) shows that after 15 days intake of metformin, female rats with induced PCOS using EV a significant reduction in serum iron, and significant increase in Zinc but serum Copper demonstrated a significant decrease compared with induced PCO non treated and control group. This finding may indicate that metformin prevent the absorption of iron from intestinal lumen leading to decrease in iron body concentration, and this finding is in agreement with other authors (Lugu *et al.,* 2007). Luca, and Francesca, (2008) have recently found that metformin increasing insulin sensitivity may decrease intestinal iron absorption in patients PCOS. Tahira *et al.,* (2011) reported that three months intake of metformin in PCOS patient caused a reduction ferritin may improve glycemic and insulin sensitivity.

Oral administration of metformin act to improvement the serum level of both Zn and Cu. It may act as antioxidant as well as improving metabolic activity in the body of the rat. Many reported found that Zn and Cu have important role in cellular metabolic regulation. Fatemah *et al.,* (2010) found positive beneficial effects on feature of metabolic syndrome in PCOS after Zn supplementation. Also, reported in large clinical study, both low consumption of dietary Zn and low serum Zn concentration were associated with increase diabetic and hypercholesterolemia. Tahira *et al.,* (2011) suggested that giving additional Zn has more benefits and effectiveness in patients with PCOS.

Pouteymour *et al.,* (2011) mentioned that Zn supplementation improve inflammatory reaction in PCOS patients. Finally, metformin could act as a regulator in cellular body led to improve metabolism and Zn absorption. PCOS is a metabolic disorder may result in dysregulation of systemic copper homeostasis. Metformin treatment improves the serum Cu concentration in PCO patients. In conclusion, the present data suggested that metformin has negative effects on iron but it was improved other minerals (Zn and Cu).

References

Akinloye O, Abbiyesuku F and Oguntibeju O. (2011). The impact of blood and seminal plasma zinc and copper concentrations on spermogram and hormonal changes in infertile Nigerian men. Reprod Biol, 11(2): Pp: 83-98.

Angeles M, Jose L, Manuel L and Hector F. (2009). Body iron stores and glucose intolerance in premenopausal women. Diabetes Care, 32(8): Pp: 1525-1530.

Bancroft JD, Stevens A and Turner DR. (1990). Theory and practice of histological techniques.3rd ed. Churchill Livingstone. : 21-226.

Chausmer A. (1998). Zinc, insulin and diabetes. J Am Coll Nutr, 17: Pp: 109-115.

Colagar A, Marzony E and Chaichi M. (2009). Zinc levels in seminal plasma associated with sperm quality in fertile and infertile men. Nutr Res, 29(2): 82-88.

Faranak S, Sahar M and Nouraddin M. (2011). High serum ferritin concentration in polycystic ovary syndrome is not related to insulin resistance. Iranian Journal of Diabetes and obesity, 3(2): 47-52.

Fatema P, Beitollah A Mahzad M and Alireza O. (2010). Effect of zinc supplementation on cardiometabolic risk factors in women with polycystic ovary syndrome. J Cardiovasc Thorac Res, 2(2): Pp: 11-20.

Gőzdemir E, kaygusuzi I and Kafali H. (2013). Is hepcidin a new cardiovascular risk marker in polycystic ovary syndrome? Gynecol Obstet Invest, 75: Pp: 196-202.

Guojun J, Long L, Ping W and Wei Z. (2004). Occupational exposure to welding fume among welders: alteration Iron, Zinc, Copper, and Lead in body fluids and the other oxidative stress status. J Occup Environ Med, 46(30): Pp: 241-248

Jacqueline A, Joan C, Kerin O, Terry D and Robert J. (2012). Prevalence of polycystic ovary syndrome a sample of indigenous women in Darwin Australia. MJA, 196: Pp; 62-66.

Luca M and Francesca P. (2008). Does metformin improve polycystic ovary syndrome symptoms through reduction in body iron stores? Eurpean Journal of Endocrinology, 10: Pp: 158-439.

Lugu M, Alvarez F, Botella J, Sanchon R, San M and Escobar H. (2007). Increased body iron stores of obese women with polycystic ovary syndrome are a consequence of insulin resistance and hyperinsulinemia and are not a result of reduced menstrual losses. Diabetes Care, 30: Pp: 2309-2313.

Mandakini P. (2005). Role of metformin in management of PCOS. J K Science.7(3): Pp: 124-127

Manuel L, Francisco A, Macarena A and Hector F. (2011). Role of decreased circulating hepcidin concentration in the iron excess of women with polycystic ovary syndrome. J Clin Endocrinol Metab, 96(3): Pp: 846-852.

Obeid O, Elfakhani M, Hais S, Iskandar M, Batal M and Mouneimne Y. (2008). Plasma Copper, Zinc and Selenium levels and correlates with metabolic syndrome components of Lebanese adult. Bio Trace Elem Res, 123: Pp: 58-65.

Pouteymour F, Alipoor B and Mehrzad S. (2011). Effect of zinc supplementation on inflammatory markers in women with polycystic ovary syndrome. Shiraz E-Medical Journal, 12(1): 30-37.

Ripudaman S and Silvio E. (2003). Metformin. Drugs, 63(18): Pp: 1879-1894. Manuel, L.; Francisco, A.; Macarena, A. and Hector, F. (2011). Role of decreased circulating hepcidin concentration in the iron excess of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 96(3): 846-852.

Saeed B, Gholam R and Frida G. (2011). Effect of metformin on serum ferritin level in women with polycystic ovary syndrome. Iran Red Crescent Med J. 13(7): 487-492.

Stefano P, Angela F, Fulvio Z and Francesco O. (2009). Evidence-Based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocrine Review. 30(1): 1-50.

Tahira D, Sidra B, Khawaja T and Fatima A. (2011). Benefits of metformin in polycystic ovarian syndrome. International Journal of Pharmacetical Sciences, 3(1): 118-124.

Takeda A. (2004). Essential trace metal and brain function. Yakugaku Zasshi. 124(9):577-585

Vandemolen D, Ratts V, Evans W, Stovall D, Kauma S and Nestler J. (2001). Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone. Fertil Steril, 75: Pp: 310-315.

Vikkorinova A, Toserova E, Krizko M and Durackova Z. (2009). Altered metabolism of copper, zinc, and magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus. Metabolism, 58: Pp:1477-1482.

Wijesekara N, Chimienti F and Wheeler M. (2009). Zinc, a regulator of Islet function and glucose homeostasis. Diabetes Obes Metab, 11: Pp: 202-214.

Yves M, Laurene P, Jacqures C, Timure G, Aygul D, Alain D and Moncef B. (2011). Zinc concentration in serum and follicular fluid during ovarian stimulation and expression of Zn+2 transporters in human oocytes and cumulus cells. Reproductive Bio Med, 10: Pp: 3-15.