Adverse Effects of Repaglinide Alone and in Combination of Vitamin E

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A B S T R A C T

The purpose of this study is to investigate the adverse effects of repaglinide. Twenty of male albino rats were randomly divided into four groups (five of each): (i) Control, (ii) Vitamin E (100 mg/kg b.wt) administered orally, (iii) Repaglinide (0.09mg/kg b.wt) administered orally, and (iv) Vitamin E plus repaglinide were administered orally at the same doses. Treatment with Vitamin E and /or repaglinide were continued for 2 successive weeks. At the end of experiment, serum samples were obtained for some biochemical analysis including: catalase (CAT), superoxidase dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA). Kidney and liver were taken for histopathological examination. The obtained results showed a significant decrease in the serum level of CAT, SOD, GPx and a significant increase in the level of MDA in repaglinide group compared to the control group. Meanwhile, the group treated with vitamin E plus repaglinide revealed an improvement in the values of antioxidant enzymes compared to rats administered repaglinide group. Liver sections showed a focal co-agulative necrosis with cell aggregation and kidney sections revealed a necrotic glomeruli with destructed hemorrhagic renal tubules in the rats treated with repaglinide. However, rats treated with both vitamin E plus repaglinide illustrated a noticed recovery of liver and kidney tissues. In conclusion, administration of vitamin E can be recommended to overcome side effects associated with repaglinide by its protective antioxidant activity.

Key words: Repaglinide, Diabetes, Antioxidants and Vit. E.

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1. INTRODUCTION

Diabetes mellitus is a serious global metabolic disorder associated mainly with a disturbance in glucose and insulin levels and associated with the development of oxidative stress and production of free radicals (Obi et al.,2016). In type 2 diabetes mellitus, the reactive oxygen species (ROS) promote apoptosis pathways in the β-cell of pancreas leading to impairment of insulin synthesis and raised insulin resistance. Consequently, there is a damaged DNA, degradation of protein and increased lipid peroxidation (Evans et al., 2006),(simmons ,2006). A report of World Health Organization has predicted a duplication in number of patients with diabetes by the year 2025, due to many defects in the pathophysiology of this disease and its complications (Ivorra et al., 1989 , WHO 2002). Anti-diabetic drugs have been used for treatment of diabetes mellitus type 2.

Most of current utilized hypoglycemic agents includes: biguanides, thiazolidinediones, meglitinides and sulfonylureas, are administered orally as monotherapy or in combination of different class for glycemic control (Obi et al., 2016). Repaglinide is a prandial glucose regulator used for organizing the elevated glucose level and belong to derivatives of carbamoylbenzoic acid. It is linked to meglitinide class of insulin secretagogues chemically and differs in action from sulfonylureas as it has a distinctive binding site at the membrane of β-cell and insulin tropic effects and a more rapid onset of action (Polonsky et al.,1988),(Scott ,2012).Pervious studies were referred to alter in the pharmacokinetic and pharmacodynamic profile of repaglinide after a powerful inhibitors or inducers administration of CYP3A4 (Hatorp et al., 2003).Therefore, changes in plasma concentrations of repaglinide due to reserve of transporter-mediated uptake and metabolism in liver (Goud et al., 2016). Furthermore, this may lead to other adverse effects associated with long use of repaglinide especially with stressed diabetic cases.

Vitamin E compounds are documented for its defensive effects against lipid peroxidation of living cells (Burton and Traber ,1990). Vitamin E has many forms: α-, β-, δ-, and γ-tocopherols and α-, β-, δ-, and γ-tocotrienols. Alpha tocopherol is the most active natural form of vitamin E ,which transfers a hydrogen atom to a different types of lipid free radical, such as carbon~centered radicals, forming α- tocopheroxyl radical which react with other free radicals, forming non-radical products (Ham and Liebler , 1997). In this study we investigated the protecting role of vitamin E against oxidative stress and damaged renal and hepatic tissues related to treatment with repaglinide using rats as animal model.

2. MATERIALS AND METHODS

2.1.Drugs

Repaglinide (NovoNorm® 1mg) manufactured by Novo Nordisk, Denmark. Vitamin E (Vitamin E® capsule) It was supplied by PHARCO pharmaceutical CO., Alex., Egypt. Vitamin E dissolved in corn oil. Every chemicals and reagents used in this study were of analytical grade.

2.2.Animals

Adult male rats from Sprague Dawley are obtained from a laboratory unit in faculty of Vet. Medicine Zagazig University (aged 10 weeks old) weighting 180 to 200 g. They were housed under standard conditions with an optimized temperature and light day cycle. The rats fed on rat chow diet and acclimatized for two weeks before beginning of the experimental study. All treatments in the current study were completed in the morning following the guidelines of laboratory animals care and the ethical strategy for the experimental animals searches (Sabir and Rocha , 2008).

2,3.Rat grouping and treatments

Twenty rat males were randomly,(5 rats of each) divided into four groups as following: Group one: rats were not received any drugs and as represented as control rats. Group two was given vitamin E (100 mg/kg b.wt) orally. Group 3 was administered orally (0.09mg/kg b.wt) of repaglinide which is the calculated therapeutic dose of the drug (Paget and Barne , 1964).Group four was given repaglinide (0.09mg/kg b.wt) with vitamin E (100 mg/kg b.wt). All the treatments were continued for successive 2 weeks.

2.4. Collection of samples

Blood samples were collected from all rats of the studied groups at the 15th day then centrifuged

at 3000 rpm for 10 min. to obtain the serum samples for some biochemical assay. After scarification of rats, liver and kidney samples were collected for histopathological examination.

2.5. Assessment of serum antioxidant markers

Catalase activity was estimated using the method described by (Aebi, 1983). Superoxidase dismutase (SOD) was estimated by the method given by (Xin and Waterman , 1991) .Glutathione peroxidase (Gpx) was estimated by the method of (Paglia and Valentine, 1967). Malondialdehyde (MDA) was estimated spectrophotometrically using method of (Varshney and Kale, 1990). All the antioxidant analysis were assayed using a Diagnostic kits.

2.6. Histopathological preparation

The selected liver and kidney prepared according to method of (Suvarna et al., 2013). Tissues were fixed in neutral formalin (10 %) solutions for 24 hrs., processing of tissues and paraffin blocks preparation were prepared then stained with hematoxylin-eosin (H&E) stains. The obtained liver and kidney sections were examined under light microscope (400x).

3.7. Statistical analysis

The obtained results were statistically analyzed using One-Way Analysis of Variance (ANOVA). The data were expressed as mean ± SD (n=5). ($p < 0.05$) was considered to be statistically significant.

3. RESULTS

The obtained results showed a significant reduction ($P < 0.05$) in values of serum catalase (CAT), superoxidase dismutase (SOD) and Glutathion peroxidase (GPx) in the group of rats treated with repaglinide only compared with control group. There was no significant difference in the same parameter between control and vit. E group. While, the group of rats treated with vit. E and repaglinide revealed a significant raise ($P < 0.05$) in values of CAT, SOD and GPx compared with group treated with repaglinide. The serum malondialdehyde (MDA) proved a significant increase ($P < 0.05$) in group (3) treated with repaglinide. Also, the group (2) treated with vit. E not revealed any difference significantly compared to the control one. There was a significant decrease ($P < 0.05$) in the group of rats administrated both (vit. E and Repaglinide) (Fig. 1).

The histopathological examination of hepatic and renal sections illustrated the changes between the studied groups. The liver of control group (a) showed typical hepatic lobular construction consisting of hepatic cords of organized hepatocytes radiating from a central vein enclosing sinusoidal network . Liver section of vitamin E-treated group (b) showing normal histological picture of hepatocytes as control rats. However, the group of rats treated with repaglinide (c) showed focal coagulative necrosis replaced by mononuclear cell aggregation and mild hepatic congestion. The liver section in group of rats administrated both repaglinide and vitamin E (d) showed mild aggregation of mononuclear cells (lymphocytes, monocytes, and plasma cells) aggregation with hepatocytes regeneration (Figure 2).

The kidney section of control group (a) showed normal renal construction of renal glomeruli and typical proximal and distal convoluted tubules. Vitamin E-treated group (b) showed normal histological picture of kidney as control. However, the group of rats treated with repaglinide (c) showed necrotic glomeruli, extensive hemorrhage and destructed renal tubules with moderate to severe congestion of renal blood vessels. The kidney section in group

of rats administrated both repaglinide and vitamin E (d) showed cellular debris in the lumen of some renal tubules with regenerated renal tubules (Figure 3).

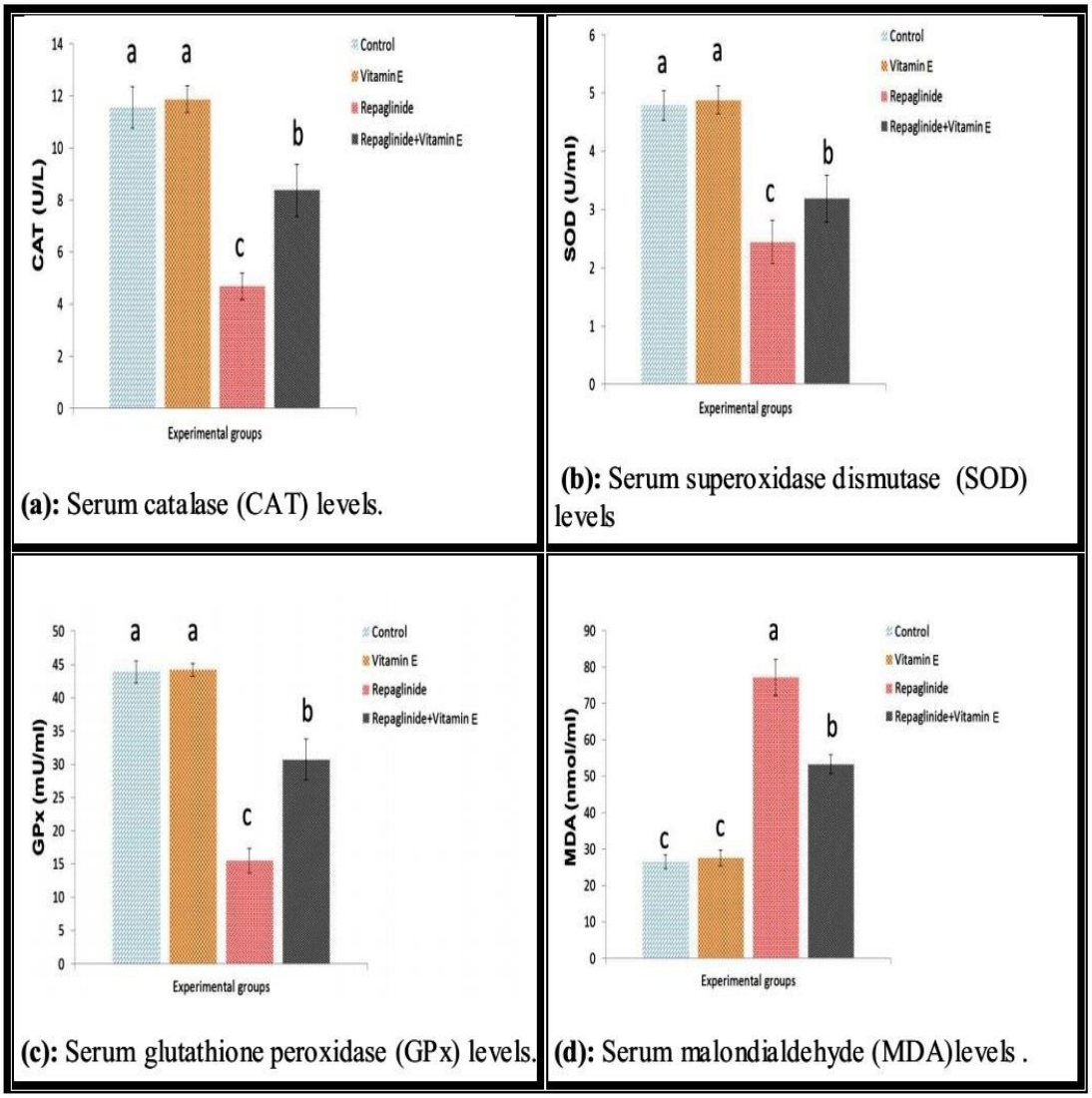

Figure (1): Effect of vitamin E and/or repaglinide on serum (a) catalase, (b) superoxidase dismutase,(c) glutathione peroxidase and (d) malondialdehyde levels of all studied groups.

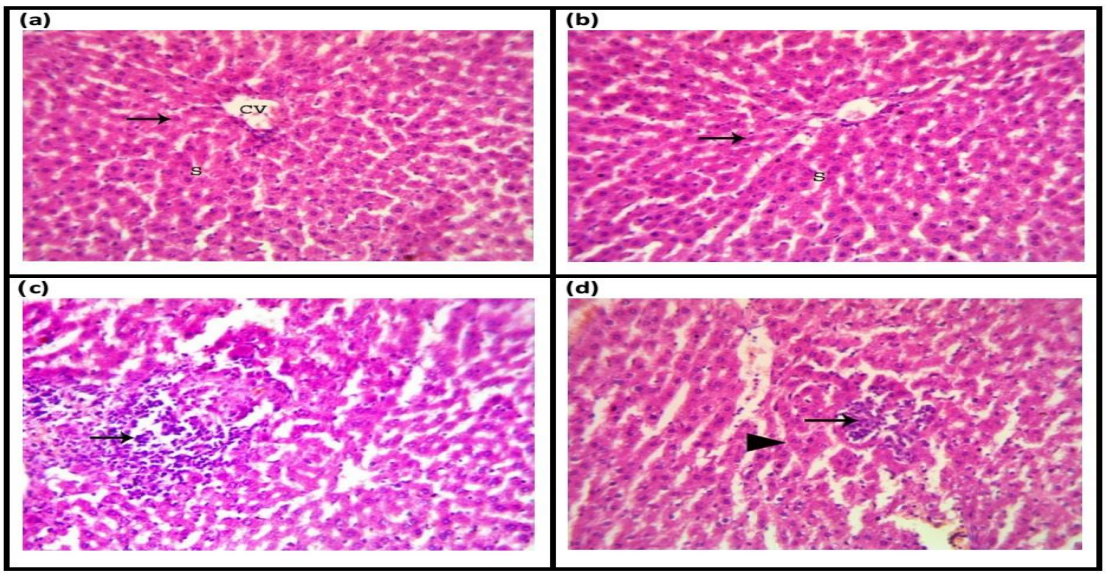

Figure (2): Histopathology examinations of rat liver sections of (a) control group, (b) Vitamin E treated group, the (arrow) refer to normal hepatocytes, (S) is sinusoidal network and (CV) is central vein. (c) Repaglinide treated group showed mononuclear cell aggregation(arrow) and (d) group treated with vitamin E and repaglinide showed hepatocytes regeneration (arrow head) (HE, 400x).

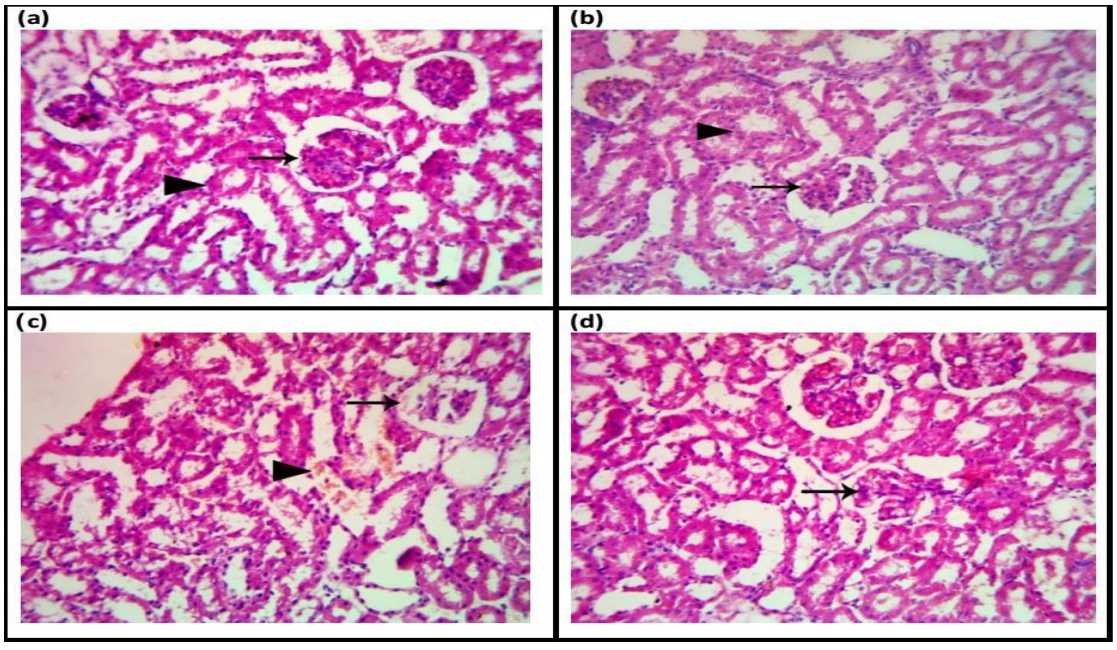

Figure (3): Histopathology examinations of rat kidney sections of (a) control group and (b) v itamin E treated group showed renal glomeruli (arrow) and tubules (arrowhead). (c) Repaglinide treated group necrotic glomeruli (arrow), extensive hemorrhage and destructed renal tubules with mild congestion of blood vessels(arrowhead).(d) group treated with vitamin E and repaglinide showing congested blood vessels (arrow) ,(HE, 400x).

4. DISCUSSION

The present study investigated the antioxidant activity of vitamin E in modulation some biochemical and histopathological changes associated with oral administration of repaglinide in adult male rats. Repaglinide is a novel short acting class of oral insulin secretagogues which stimulates insulin release from beta cells of pancreas by binding to and ultimate ATP dependent potassium channels (Goud et al., 2016).The attenuation of natural antioxidant mechanism by oxidative stress causes augment in Reactive oxygen species (ROS-) causing oxidative damage implicated in many metabolic disorders as diabetes (El-Demerdash et al., 2005),(Pitocco,et al.,2010). The system of antioxidants including: enzymatic systems, glutathione and vitamins A, C, and E (Aksoy ,et al.,2005). Catalase (CAT) and superoxide dismutase (SOD) acting as a free radical scavenging enzymes for preserve biological system from oxidative damage (Del Rio et al., 2005).

The obtained results showed a significant reduction in the level of antioxidant enzymes of the group of rats treated with repaglinide only ,While a significant increase of the antioxidant markers were noticed in the group treated with vit. E and Repaglinide. Several studies confirmed that the increased level of CAT and SOD is an indication of their ability to scavenge ROS, thus contributing to the defense action against oxidative stress and preventing additional injure to membrane lipids (Onyeka et al., 2013). Glutathione is a non enzymatic antioxidant that acts synergistically potentially to scavenge ROS by conserving oxidative system within the cells and tissue structures (Ahmed et al., 2011). It has been stated an alternation in the primary enzymatic antioxidant including a decreasing in (CAT, SOD and Gpx) and an elevated levels of lipid peroxidation ensuring the incidence of oxidative stress especially in diabetic rats (Bhor et al., 2004).

Results comparable to the present study were observed that vitamin E encouraged a decline in the indicators of oxidative stress and protein glycation (Reunanen et al., 1998). A previous data about treatment with vit. E in rats leading to drop in the increased level of lipid peroxidation and enhance in level of superoxide dismutase activity (Shirpoor et al., 2007). Lipid peroxidation of unsaturated fatty acids is frequently used as an indicator for oxidative damage. It causes cell membrane dysfunction by peroxidation impairment of cell membrane fluidity and enzyme binding receptors (Halliwell , 2000). The increased lipid peroxidation during diabetes as found in the increased concentration of malondialdehyde (MDA), an end step of lipid peroxidation (Rauscher et al., 2000). Metformin and glibenclamide have ensured ability to reduce MDA level in the treated diabetic rats confirmed by (Onyeka et al., 2013). However, Repaglinide may have some protective effect against lipid peroxidation.

The obtained results were confirmed by the histopathological changes in the kidney and liver sections among the studied group. There is co-agulative necrosis of the hepatic tissues with mononuclear cell aggregation of liver. In addition, a hemorrhagic renal tubules and a necrotic glomeruli of kidney in the group of rats treated with repaglinide only. The co-administration of vit. E with repaglinide revealed a noticed improvement in the architecture of hepatic and renal tissue with a mild leukocytic infiltration which may be due to the protective antioxidant activity of vitamin E. A parallel study applied on liver and kidney sections from diabetic guinea pig treated with repaglinide for 4 weeks showed most of histological structures of

kidney still normal like in the control group, while liver section showed dilated central vein and moderate centrizonal necrosis of the hepatocytes(Abd Alsalaam,et al.,2016). It had been reported the improvement of the pathological changes in the liver and kidney tissues after using vitamin E (Boskabady et al., 2012).

5. CONCLUSION

Co- administration of repaglinide and vitamin E exerts a significant antioxidant activity in rats, thus causal to the protective effect against oxidative stress-induced damage during the treatment with repaglinide.

6. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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