

Antioxidative effect of metformin on valproic acid induced hepatotoxicity in male rats

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Abstract:

Metformin is 1,1-dimethylbiguanide hydrochloride, is the first-line therapy for type 2 diabetes. Additionally, several studies focused on the role of metformin in antioxidant activities for the treatment of hepatic disorders. The experimentally

-based result on valproic acid's liver injury, a front-line medicine for the treatment of epilepsy, attracted a lot of interest. As a result, the effect of metformin on valproic acid-induced redox disturbances in rat hepatic tissue was studied. metformin at 250 mg/kg dose was administered via oral gavage for 30 days, and valproic acid at a dose of 400 mg/kg was administered by intraperitoneal route starting from the twenty-second day of the experiment, for eight days to induce hepatotoxicity. Treatment with metformin reduced valproic acid-enhancing alanine aminotransferase, aspartate aminotransferase activities. Tissue levels of malondialdehyde in the liver tissue of valproic acid-treated rats significantly increased (P -value < 0.05) whereas glutathione decreased. The coadministration of metformin with valproic acid significantly decreased the malondialdehyde levels and increased glutathione levels (P -value < 0.05). Finally, metformin protected rats from valproic acid-induced hepatotoxicity, improved antioxidant status, and reduced hepatic oxidative stress.

Key words: Metformin, valproic acid, alanine aminotransferase, aspartate aminotransferase malondialdehyde, glutathione.

التأثير المضاد للأوكسدة للميتفورمين على السمية الكبدية التي يسببها حمض الفالبرويك في ذكور الجرذان

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الخلاصة:

الميتفورمين هو 1،1-ثنائي ميثيل بيجوانيد هيدروكلوريد ، وهو علاج الخط الأول لمرض السكري من النوع الثاني. بالإضافة إلى ذلك ، ركزت العديد من الدراسات على دور الميتفورمين في الأنشطة المضادة للأوكسدة لعلاج الاضطرابات الكبدية. جذب الاكتشاف القائم على التجارب حول إصابة الكبد لحمض الفالبرويك ، وهو دواء من الخطوط الأمامية لعلاج الصرع ، الكثير من الاهتمام. نتيجة لذلك ، تمت دراسة تأثير الميتفورمين على اضطرابات الأوكسدة التي يسببها حمض

الفالبرويك في الأنسجة الكبدية للفئران. تم إعطاء الميتفورمين بجرعة 250 مجم / كجم عن طريق الفم عن طريق التزقيم الفموي لمدة 30 يومًا ، وتم إعطاء حمض الفالبرويك بجرعة 400 مجم / كجم عن طريق الصفاق بدءًا من اليوم الثاني والعشرين من التجربة ، لمدة ثمانية أيام للحث على السمية الكبدية. قلل العلاج بالميتفورمين من نشاط حمض الفالبرويك المتزايد للأنين أمينوترانسفيراز و أسبارتات أمينوترانسفيراز. زادت مستويات مالونديالدهيد في أنسجة الكبد للفئران المعالجة بحمض الفالبرويك بشكل ملحوظ (قيمة $p < 0.05$) بينما انخفض الجلوتاثيون. أدى تناول المتزامن للميتفورمين مع حمض الفالبرويك إلى انخفاض كبير في مستويات مالونديالدهيد وزيادة مستويات الجلوتاثيون (قيمة $p < 0.05$). أخيرًا ، قام الميتفورمين بحماية الفئران من السمية الكبدية التي يسببها حمض الفالبرويك ، وتحسين حالة مضادات الأكسدة ، وتقليل الإجهاد التأكسدي الكبدية.

الكلمات المفتاحية: ميتفورمين ، حمض فالبرويك ، الأنين أمينوترانسفيراز ، أسبارتات أمينوترانسفيراز مالونديالدهيد ، جلوتاثيون

Introduction

Many successful modern-day medications for neurological illnesses have off-target toxicity, which is a serious worry for some patients receiving therapy, since some pharmaceuticals have harmful effects on important organs that express their metabolizing enzymes in add to their supposed therapeutic effects [1]. The valproic acid (V.A), also known as 2-propylpentanoic acid, is a first-line synthetic medication used to treat a variety of neurological disorders, including epilepsy, bipolar disorder, and migraine [2]. On the other hand, V.A has been shown to increase hepatic damage markers in humans and experimental rat models [3]. The reactive metabolites also hypothesized to play a crucial part in the causation of their hepatotoxicity due to oxidative stress caused by excess production of reactive species. The valproic acid is metabolized by cytochrome P450 to the 2, 4-diene-VPA, which is an indicator of oxidative stress in the liver linked to necrotic cell death and hepatotoxicity [4]. Since V.A treatment has been linked to oxidative stress via a dose-dependent elevated in serum and hepatic levels of endogenous lipid peroxidation (LPO) indicators [5], it's critical to find a novel medicinal agent capable of counteracting its hepatic injury effects while preserving the desired therapeutic outcomes. Metformin, also known as 1,1-dimethylbiguanide hydrochloride, is the first-line treatment for type 2 diabetes [6]. It demonstrated antioxidant and hepatoprotective properties

in several experimental models via a variety of mechanisms [7]. Metformin inhibition of mitochondrial complex I has been found, and the complex I may play a key role in the generation of cellular ROS, resulting in increased lipid peroxidation and MDA levels. It is widely known that inhibiting this complex with metformin lowered ROS production and lipid peroxidation, resulting in lowers MDA and higher GSH levels [8], [9]. The metformin has been found to protect against hepatotoxicity caused by methotrexate [10], ethanol [11], and other chemicals. However, the hepatoprotective effect of metformin on V.A-induced hepatic injury has not been explored. As a result, this study looked at metformin protective properties against V.A-induced hepatotoxicity, as well as the mechanisms behind them.

Materials and methods

Chemicals and reagents

Valproic acid (Sigma-Aldrich St Louis, MO, USA), metformin (Pioneer Pharmaceutical Company), distilled water (PDPL; India).

Experimental animals

Twenty-four albino rats (males) with weight range 150 g-220g were used in current research. These rats were brought and acclimatized from animal house of the College of Pharmacy/ Mustinsiriyah University for 10 days. They were housed in research plastic cages indimention (20x25x35 cm) and provided pellets and water.

Treatments and animal grouping

This study employed a simple randomized approach. After ten days of an adaptation time, these rats were divided randomly into four groups (six rats for each). group 1: negative control: rats were given 1 ml of distilled water by oral gavage for 30 days, group 2: rats received metformin (250 mg/kg/day for 30 days) via oral gavage, group 3: rats received V.A (400 mg/kg) starting from the 22nd day of the experiment for eight days to induce hepatotoxicity by intraperitoneal route, groups 4: animals were received metformin orally via gavage at doses of 250 mg/kg/day for 30 days and valproic acid (400 mg/kg) starting from the 22nd day of the experiment for 8 days by intraperitoneal route. The doses of metformin were chosen according to human equation dose and previous studies [11], and V.A dose determination depended on a preliminary study.

Collection of serum and liver samples

On day 31, the animals were anesthetized and injected intraperitoneally with 50 mg/kg ketamine and 5 mg/kg xylazine [12]. The blood was collected from the right ventricle of the heart and placed in gel tubes, and centrifuged at 2500 rpm for 15 minutes for serum separation [13]. The serum was collected in an Eppendorf for the estimation of liver enzymes. Whereas tissue slices from the liver were collected and homogenized in 0.01 phosphate buffer solution for the estimation of tissue malondialdehyde and glutathione contents.

Determination of liver function

alanine aminotransferase (ALT) (Genway Biotech, Inc), aspartate aminotransferase (AST) (Sigma-Alorich), levels in serum samples were measured by colorimetric kits according to the manufacturer's guide.

Determination of oxidative stress biomarkers in liver tissue homogenate

The level of oxidative stress was determined by measuring the levels of

reduced glutathione (GSH), and malondialdehyde (MDA) in liver homogenate using sandwich ELISA kit (MyBioSource) according to manufacturing procedure. Rat GSH or MDA present in the sample or standard binding to antibody on wells. Then added a biotinylated detection antibody specific and binds to rat GSH or MDA captured by the first antibody. Then washed well to remove unbound antigen, following Horseradish Peroxidase is added and bound to the biotin-conjugated antibody, The washing of unbound ingredients was done. Then the substrate was injected to each well and the blue color appeared. The stop solution was then added to finish the enzyme-substrate reaction and the yellow color appeared. The wavelength is 450 nm.

Statistical analysis

Data analysis was accomplished via the use of Statistical Packages for Social Sciences (SPSS) (version 25) software. The descriptive statistics were reported as mean \pm standard error of mean (SEM). One-way Analysis of Variance (ANOVA) test was used to verify the significance of the difference between the eight studied groups, followed by the Tukey test. A P-value is considered nonsignificant if it is P-value > 0.05 and significant if it is P-value < 0.05 .

Results

Influence of metformin on valproic acid-induced changes on serum liver enzymes of rats

Results obtained in group3 showed that administration of V.A revealed significant increased (p-value < 0.05) ALT, and AST levels in serum to (204 and 274 nmol/ml) respectively; in comparison to control animals. In contrast, group 4 that treatment with metformin showed a significant decrease in serum levels of ALT, and AST when compared to G3 (P-value < 0.05), and non-significant differences in ALT level when compared to G1 (P-value > 0.05) but it significantly rose when

compared with G2 (P-value < 0.05) while AST level remained significantly different when compared to G1 and G2 (P-value < 0.05). as shown in table (1), and figure (1).

Table (1): levels of liver enzymes (ALT, AST) in all groups.

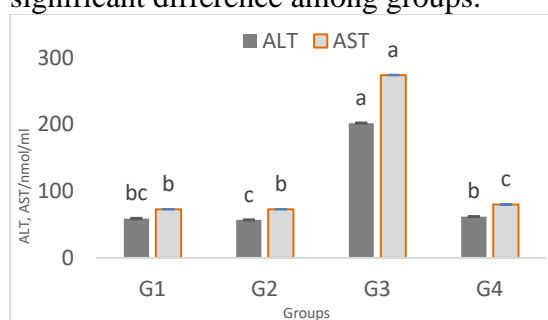
Groups	N	ALT nmol/ml	AST nmol/ml
G1	6	59 ± 1 bc	73 ± 0.08b
G2	6	57 ± 0.7 c	73 ± 0.08b
G3	6	202 ± 0.8 a	274 ± 0.4a
G4	6	62 ± 0.3 ^b	80 ± 0.6c

Each value is given as the mean± SEM. The statistical analysis was done by using one-way ANOVA followed by the Tukey test. G1: negative control group, G2: 250mg/kg metformin group, G4: positive control group (400mg/kg) V.A, G4:250mg/kg metformin + V.A, G8: 500mg/kg metformin + V.A. Different lower – case

Table (2): GSH and MDA levels in all groups

Groups	N	GSH (µg /ml)	MDA (nmol/ml)
G1	6	16.5 ± 0.8	0.76 ± 0.02
G2	6	17.4 ± 1	0.97± 0.04
G3	6	6.3 ± 0.6	4.9±0.3
G4	6	14.8 ± 0.7	1.3 ± 0.2

letters in same column indicate a significant difference among groups.



Figures (1): Change in levels of liver enzymes (ALT, AST) in all groups

The results represented as mean ± SEM. The statistical analysis was done by using one-way ANOVA followed by the Tukey test. G1: negative control group, G2:250mg/kg metformin group, G3: positive control group (400mg/kg V.A), G7: 250mg/kg metformin +

V.A, G4: 500mg/kg metformin + V.A. Different lower – case letters in same column indicate a significant difference among groups.

Effect of metformin on V.A – induced oxidative stress in the liver tissue of rat

Group 3 showed a significant decreased (p-value < 0.05) in GSH levels to (6.3 µg /ml) when compared to group1 and group 2 while, GSH level in G4 was a significantly increased (14.8 ± 0.7 µg /ml) when compared with G3 (p-value < 0.05) and non-significantly differences when compared to G1 and G3 (P-value < 0.05). group 3 showed a highly significant increased (p-value < 0.05) in lipid peroxidation (MDA) in hepatic tissue when compared to group1 and group 2. G4 had a significantly decreased MDA level (1.3 ± 0.21 nmol/ml) when compared with G3 (p-value < 0.05) and non-significantly difference when compared to G1 and G3 (P-value > 0.05) as shown in table (2), and figure (2).

Each value is given as the mean± SEM. The statistical analysis was done by using one-way ANOVA followed by the Tukey test. G1: negative control group, G2: 250mg/kg metformin group, G3: positive control group (400mg/kg V.A), G4: 250mg/kg metformin + V.A. Different lower – case letters indicate a significant difference among groups.

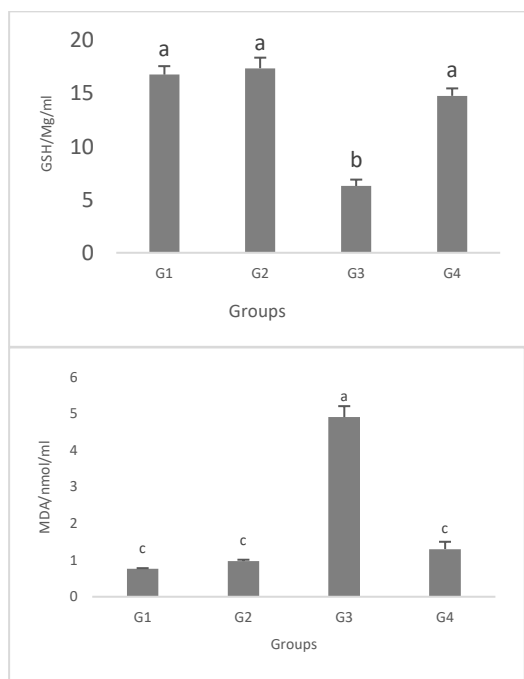


Figure (2): Change GSH and (MDA) levels in all groups.

The results represented as mean \pm SEM. The statistical analysis was done by using one-way ANOVA followed by the Tukey test. G1: negative control group, G2: 250mg/kg metformin group, G3:(400mg/kg V.A), G4: 250mg/kg metformin + V.A. Different lower – case letters indicate a significant difference among groups

Discussion

In this study, valproic acid dose used was produced a significant increase in ALT, AST serum levels when compared to control and metformin group as shown in table (1) and figure (1). These findings can be attributed to cell necrosis, which increases the leakage of enzymes from hepatocytes. The findings are in line with Shakya *et al.* (2018) [14]. Metformin in combination with V.A significantly lowers abnormally elevated serum levels of ALT when compared with the control and metformin groups and decreases AST levels, but their remained significant differences when compared with the control and metformin group. This suggests that metformin decreases cell necrosis in the liver of rats after valproic

acid overdose. Previous research has focused on the possible protective impact of metformin and lowered serum levels of liver enzymes against hepatotoxicity caused by various drugs such as thioacetamide. (Al-hashem *et al.* 2018), and methotrexate (Risk *et al.* 2018) [10]. The tissue level of GSH was significantly decreased (p -value < 0.05) in group 3 as shown in Table 2 and Figure (2) and then returned to normal levels in group 4 when compared with groups 1, and 2. Whereas the tissue level of MDA was significantly elevated (p -value < 0.05) in group 3 when compared with the group 1 and group 2 and approximately returned to normal in group 4.

These results were in line with several previous studies like Abdelkader *et al.* (2020) [1], (Omidipour *et al.* 2021) [15], Ola *et al.* 2021 [16], and Tong *et al.* 2005 [17] which have shown that chronic VPA administration has been attributed to hepatotoxicity by the generation of (ROS) as a result of CYP2E1 metabolic activation and initiating or increasing oxidative stress indicators as evidenced by activation of lipid peroxidation, which results in higher MDA levels and a reduction in GSH levels. This causes lysosomal membrane leakage and a decrease in the ability to defend against oxidative stress, resulting in cellular damage. Metformin and V.A coadministration improved the antioxidative system independent of glycemic control. Metformin has a direct scavenging impact on ROS, which helps to restore the antioxidant system and reduce oxidative stress [18]. Several previous studies have been focused on the antioxidant effect of metformin in lowering oxidative damage markers such as Rizk *et al.* (2018) [10], Kelly *et al.* (2015) [9], Al-Hashem *et al.* (2018) [19] and Borole *et al.* (2016) [11], have found metformin inhibits mitochondrial complex I, which may play an important role in the generation of cellular ROS, resulting in increased lipid peroxidation and raised MDA levels. It is widely understood that

blocking this complex with metformin reduced ROS production^{[20],[21]} and consequent lipid peroxidation, resulting in decreased MDA and elevated GSH levels.

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